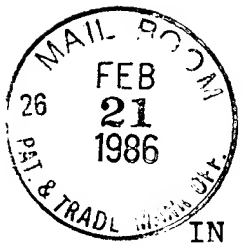
#15



IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re: U.S. Patent 4,263,432
Issued: April 21, 1981
To: Masaru Iwanami et al.
Assignee: Yamanouchi Pharmaceutical Co., Ltd.
For: 7α-Methoxy-7β-(1,3-dithietane-2-carboxamido)cephalosporanic Acid Derivatives

Date: Feb. 21, 1986

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D.C. 20231

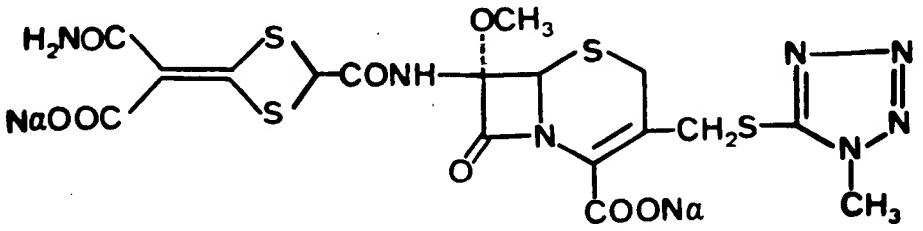
Sir:

APPLICATION FOR EXTENSION OF PATENT TERM
UNDER 35 U.S.C. 156

The Applicant is Yamanouchi Pharmaceutical Co., Ltd., a Japanese corporation (hereinafter "Yamanouchi"). Yamanouchi represents that it is the owner and assignee of the entire interest in and to letters patent of the United States No. 4,263,432 granted to Masaru Iwanami, Tetsuya Maeda, Yoshinobu Nagano, Masaharu Fujimoto, Noriaki Nagano and Atsuki Yamazaki on April 21, 1981 by virtue of an assignment to Yamanouchi recorded June 7, 1978, Reel 3556, Frame 870; Applicant acting through its duly authorized agent, ICI Americas Inc., a Delaware corporation, and the undersigned attorney hereby submits this application for extension of patent term under 35 U.S.C. 156 by providing the following information as required by the guidelines published by the U.S. Patent and Trademark Office. Agent's status as agent for

Yamanouchi is evidenced by an Authorisation of Agent and Power of Attorney, the originally signed document being attached hereto as Attachment A.

(1) The approved product is "Cefotan®" (cefotetan disodium) which is the disodium salt of [6R-(6α,7α)]-7-[[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl]carbonyl]-amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid and has the following structure:



A professional information brochure is attached hereto as Attachment B.

(2) The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act, 21 U.S.C. 355, Sections 505 and 507.

(3) Cefotan® (cefotetan disodium) received permission for commercial marketing or use under Section 507 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 355) on December 27, 1985.

(4) This application for extension of patent term under 35 U.S.C. 156 is being submitted within the sixty (60) day period permitted for submission, the last day for said submission being February 26, 1986.

(5) The complete identification of the patent for which an extension is being sought is as follows:

Inventors: Masaru Iwanami, Tetsuya Maeda, Yoshinobu Nagano, Masaharu Fujimoto, Noriaki Nagano and Atsuki Yamazaki
Patent Number: 4,263,432
Issue Date: April 21, 1981

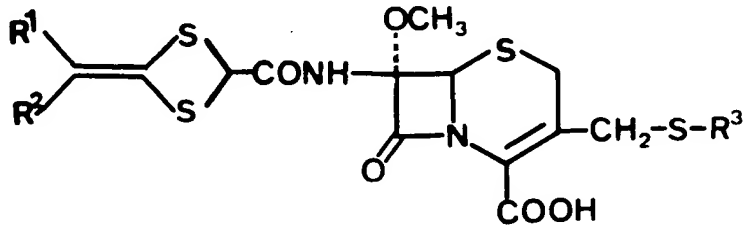
(6) A copy of the patent for which an extension is being sought is attached herewith in the form specified in the guidelines of the United States Patent and Trademark Office as Attachment C.

(7) No disclaimer, certificate of correction, receipt of maintenance fee payment, or reexamination certificate has been issued with respect to U.S. Patent No. 4,263,432.

(8) U.S. Patent 4,263,432 claims compounds having a generic structure which includes the approved human drug product as follows:

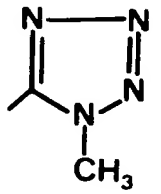
Claim 1 reads:

A 7α-Methoxy-7β-(4-substituted methylene-1,3-dithietane-2-yl)carboxamido-3-heterocyclic thiomethyl-Δ^3-cephem-4-carboxylic acid represented by the formula



wherein R^1 represents a carboxyl group or a derivative thereof selected from the group consisting of lower alkyl, phenyl lower alkyl and naphthyl lower alkyl esters of said carboxyl group and carbamoyl, carbazoyl, and cyano groups; R^2 represents a hydrogen atom, a lower alkyl group, a lower alkoxy group, $R^4S(O)_n$ group wherein R^4 represents a lower alkyl group and n represents 0, 1 or 2, a lower alkanoyl group, a phenyl group, a naphthyl group, a benzoyl group, a naphthoyl group, a R^1 group, a lower alkenyl group, a sulfamoyl group, a pyridyl group or a thiadiazolyl group; and R^3 represents a lower alkyl-substituted tetrazolyl group or a lower alkyl-substituted thiadiazolyl group and the pharmaceutically acceptable salts thereof.

The approved human drug product Cefotan® (cefotetan disodium) is a pharmaceutically acceptable salt (namely, the disodium salt) of a compound of the formula shown in Claim 1 wherein R^1 is H_2NOC- (carbamoyl); R^2 is a carboxyl group (a R^1 group) from which a sodium salt has been formed to have R^2=NaOOC-; R^3=



(a lower alkyl-substituted tetrazolyl group); and the acid group at the 4 position has also been formed as the sodium salt.

(9) The relevant dates and information pursuant to 35 U.S.C. 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(a) U.S. Patent 4,263,432 was issued on April 21, 1981;

(b) Application for investigational new drug exemption ("IND") for Cefotan® (cefotetan disodium)* was filed on September 4, 1981 and became effective October 4, 1981, IND 19,216;

(c) New drug application ("NDA") for Cefotan® (cefotetan disodium) was submitted on January 20, 1984 as NDA 50-588;

(d) NDA 50-588 was considered as withdrawn and resubmitted on April 10, 1985, due to a substantial amendment for use as a prophylactic anti-infective agent prior to surgery;

---

*Note that the product went through several brand name changes during the regulatory review period; these names are Cefotetan (which is now the generic name), Apacef® and finally Cefotan®.

(e) NDA 50-588 for Cefotan® (cefotetan disodium) was approved on December 27, 1985.

(10) A brief description of the activities undertaken by the Applicant through its agent ICI Americas Inc. during the applicable regulatory review period with respect to Cefotan® (cefotetan disodium) and the significant dates applicable to such activities is attached hereto as Attachment D.

(11) Applicant is of the opinion that U.S. Patent 4,263,432 is eligible for extension under 35 U.S.C. 156 because it satisfies all the requirements for such extension as follows:

(a) 35 U.S.C. 156(a)
U.S. Patent 4,263,432 claims a human drug product;

(b) 35 U.S.C. 156(a)(1)
The term of U.S. Patent 4,263,432 has not expired before submission of this application for extension;

(c) 35 U.S.C. 156(a)(2)
The term of U.S. Patent 4,263,432 has never been extended;

(d) 35 U.S.C. 156(a)(3)
The application for extension is submitted by the agent for the owner of U.S. Patent 4,263,432;

(e) 35 U.S.C. 156(a)(4)
The product, Cefotan® (cefotetan disodium), has been subject to a regulatory review period before its commercial marketing or use;

(f) 35 U.S.C. 156(a)(5)(A)
The permission for the commercial marketing or use of the product, Cefotan® (cefotetan disodium), after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Federal Food, Drug, and Cosmetic Act (21

U.S.C. 355), under which such regulatory review period occurred; and

(g) 35 U.S.C. 156(c)(4)

No other patent has been extended for the same regulatory review period for the product Cefotan® (cefotetan disodium).

The length of extension of the patent term of U.S. Patent 4,263,432 claimed by applicant is two years minus 132 days, the maximum possible under 35 U.S.C. 156(g)(4)(C) with the limit imposed by 35 U.S.C. 156(c)(3), since the patent involved was issued before the date of enactment of Section 156, an exemption under subsection (i) of Section 505 and an application under Section 507 were submitted with respect to the approved product before the date of enactment of Section 156, and the commercial marketing or use of the product was not approved before the date of enactment of Section 156. The regulatory review period exceeds two years as shown by the following:

(a) The regulatory review period under 35 U.S.C. 156(g)(1)(B) was from October 4, 1981 until December 27, 1985, which is 1544 days or 4.2 years.

(b) The period of review, "Testing Period", under 35 U.S.C. 156(g)(1)(B)(i) was from October 4, 1981 (effective date of IND) until January 20, 1984 (NDA submission date), which is 838 days or 2.3 years.

(c) The period of review, "Application Period", under 35 U.S.C. 156(g)(1)(B)(ii) was from January 20, 1984 (NDA submission date) until December 27, 1985 (NDA approval date), which is 706 days or 1.9 years.

(d) The total regulatory review period under 35 U.S.C. 156(g)(1)(B) (4.2 years) is subject to the limitation of 35 U.S.C. 156(g)(4)(C) and therefore the period of extension determined on the basis of the regulatory review period, as determined under 35 U.S.C. 156(g)(1)(B), may not exceed two years.

(e) Under 35 U.S.C. 156(c)(2) the period of extension includes only one-half of the period determined under 35 U.S.C. 156(g)(1)(B)(i) i.e., 419 days or 1.1 years. In the absence of

the limitation noted above, the permissible period of extension for U.S. Patent No. 4,263,432 would have been 1125 days (419 plus 706) or 3.1 years.

(f) In compliance with 35 U.S.C. 156(c)(3) the period remaining in the term of U.S. Patent No. 4,263,432 after NDA approval of Cefotan® (cefotetan disodium) (i.e., from December 27, 1985 to April 21, 1998) is 4502 days or 12.3 years, which, when added to the two year period of extension claimed in this application is 5232 days or 14.3 years and therefore is 132 days or .36 year in excess of fourteen years.

(g) The term for which extension of U.S. Patent 4,263,432 is sought is thus calculated to be 2 years minus 132 days which is 598 days or 1.64 years.

(12) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to any determination to be made relative to this application for extension.

(13) A check for the prescribed fee for receiving and acting upon this application for extension is enclosed herewith. Authorization is hereby given to charge any additional fees due or credit any overpayment to Applicant's Deposit Account No. 0505412-09-0011. This application is submitted in duplicate. The requisite declaration set forth in paragraph (c) of Section D of the guidelines for extension of patent term under 35 U.S.C. 156 is also attached hereto.

Respectfully submitted,

ICI AMERICAS INC.

By Rosemary M. Miano
Rosemary M. Miano
Agent for Applicant
Registration No. 29,674

2/21/86

RMM:sms
ICI AMERICAS INC.
Patents Section - Law Dept.
Wilmington, DE 19897
(302) 575-3729

CERTIFICATION

The undersigned hereby certifies that this application for extension of patent term under 35 U.S.C. 156 including its attachments and supporting papers is being submitted as duplicate originals.

Date Feb. 21, 1986

Rosemary M. Miano
ROSEMARY M. MIANO

022186CLJ01

U.S. PATENT NO. 4,263,432

Title: 7α-Methoxy-7β-(1,3-dithietane-2-carboxamido)cephalosporanic Acid Derivatives

Inventor(s): Masaru Iwanami et al

I hereby certify that this correspondence, including a Declaration, Application for Extension of Patent Term Under 35 U.S.C. 156 (in triplicate) which includes Attachment A (an Authorization of Agent & Power of Attorney), Attachment B (a professional information brochure on CEFOTAN®), Attachment C (U.S. Patent 4,263,432 in duplicate) and Attachment D (Chronology of Regulatory Activities for CEFOTAN®), a post card and $750.00 check is being mailed under 37 CFR 1.10 with the Express Mail Post Office to Addressee Service, Label No. B80892821 addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231 on this 21st day of February, 1986 by

Herbert C. Griffith
(Name of person mailing paper)

(Signature of person mailing paper)

Chief Patent Counsel
ICI Americas Inc.
Wilmington, Delaware 19897

(302) 575-3721
U.S. Patent 4,263,432

U.S. PATENT NO. 4,263,432

#5

Title: 7α-Methoxy-7β-(1,3-dithietane-2-carboxamido)cephalosporanic Acid Derivatives

Inventor(s): Masaru Iwanami et al

attachments not in file (except C)

I hereby certify that this correspondence, including a Declaration, Application for Extension of Patent Term Under 35 U.S.C. 156 (in triplicate) which includes Attachment A (an Authorization of Agent & Power of Attorney), Attachment B (a professional information brochure on CEFOTAN®), Attachment C (U.S. Patent 4,263,432 in duplicate) and Attachment D (Chronology of Regulatory Activities for CEFOTAN®), a post card and $750.00 check is being mailed under 37 CFR 1.10 with the Express Mail Post Office to Addressee Service, Label No. B80892821 addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231 on this 21st day of February, 1986 by

Herbert C. Griffith
(Name of person mailing paper)

Herbert C. Griffith
(Signature of person mailing paper)

Chief Patent Counsel
ICI Americas Inc.
Wilmington, Delaware 19897

(302) 575-3721

U.S. Patent 4,263,432

02/26/86 4263432 1 111 750.00 CK

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re: U.S. Patent 4,263,432
Issued: April 21, 1981
To: Masaru Iwanami et al.
Assignee: Yamanouchi Pharmaceutical Co., Ltd.
For: 7α-Methoxy-7β-(1,3-dithietane-2-carboxamido)cephalosporanic Acid Derivatives

Date: [illegible]

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D.C. 20231

Sir:

DECLARATION

The undersigned, Agent for Applicant Yamanouchi Pharmaceutical Co., Ltd. (a Japanese corporation), and attorney for Agent ICI Americas Inc. (a Delaware corporation, having a place of business at Concord Pike and Murphy Road, Wilmington, Delaware 19897), for extension of patent term under 35 U.S.C. 156 with respect to U.S. Patent No. 4,263,432 hereby declares that:

(1) She has reviewed and understands the contents of the Application for Extension of Patent Term being submitted pursuant to 35 U.S.C. 156 and Section D of the guidelines for extension of patent term under 35 U.S.C. 156;

(2) She believes the patent is subject to extension pursuant to 35 U.S.C. 156 and Section A of the guidelines for extension of patent term under 35 U.S.C. 156;

(3) She believes an extension of the length claimed in the Application is fully justified under 35 U.S.C. 156; and

(4) She believes the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 35 U.S.C. 156 and Section B of the guidelines for extension of patent term under 35 U.S.C. 156.

The undersigned hereby declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any extension of patent term issuing thereon.

Date 4-21-1986

ROSEMARY M. MIANO
Reg. No. 29,674

ICI AMERICAS INC.
Patents Section - Law Dept.
Wilmington, DE 19897
(302) 575-3729

022186CLJ02

ATTACHMENT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re an application for extension of the term of US Patent No. 4,263,432 under the Drug Price Competition and Patent Term Restoration Act of 1984

AUTHORISATION OF AGENT AND POWER OF ATTORNEY

We, Yamanouchi Pharmaceutical Co. Ltd., of No.1-8, Azusawa 1-chome, Itabashi-ku, Tokyo 174, Japan, being the owner of record of US Patent No. 4,263,432, hereby authorise and appoint ICI Americas, Inc., a corporation organised and existing under the laws of the State of Delaware and having offices at Concord Pike and New Murphy Road, Wilmington, Delaware 19897, USA and the Patent Attorneys named below:

Rosemary Milton Miano (Reg.No. 29,674)
John Michael Sheehan (Reg.No. 26,065)
Charles Albert Haase (Reg.No. 22,675)

all being employees of the said ICI Americas, Inc.; individually and collectively to be our agents and attorneys for the purpose of the above application and to transact all business in the Patent and Trademark Office in connection therewith.

And we request that all communications in the above matter be sent to the following address:

Patents Section
Law Department
ICI Americas, Inc.,
Concord Pike and New Murphy Road,
Wilmington
Delaware 19897
U.S.A.

Signed by

Shigeo Morioka

Name: Shigeo Morioka
Position: President
Authorised Officer
Yamanouchi Pharmaceutical Co. Ltd.

Date: February 5, 1986

PROFESSIONAL INFORMATION BROCHURE

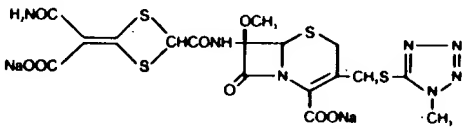
CEFOTAN™
(cefotetan disodium)

For Intravenous or Intramuscular Use

DESCRIPTION

CEFOTAN™ (cefotetan disodium) is a sterile, semisynthetic, broad-spectrum, beta-lactamase resistant, cephalosporin (cephamycin) antibiotic for parenteral administration. It is the disodium salt of [6*R*-(6α, 7α)]-7-[[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl]carbonyl]amino]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-8-oxo-5-*thia*-1-*azabicyclo*[4.2.0]oct-2-ene-2-carboxylic acid. Its molecular formula is $C_{17}H_{15}N_7Na_2O_8S_4$ with a molecular weight of 619.56.

STRUCTURAL FORMULA



CEFOTAN™ contains approximately 80 mg (3.5 mEq) of sodium per gram of cefotetan activity. It is a white to pale yellow powder which is very soluble in water. The solution varies from colorless to yellow depending on the concentration. The pH of freshly reconstituted solutions is usually between 4.5 to 6.5.

CLINICAL PHARMACOLOGY

High plasma levels of cefotetan are attained after intravenous and intramuscular administration of single doses to normal volunteers.

PLASMA CONCENTRATIONS AFTER 1.0 GRAM IV* or IM DOSE

Mean Plasma Concentration (μg/mL)

	Time After Injection					
Route	15 min	30 min*	1 h	2 h	4 h	8 h	12 h
IV	92	158	103	72	42	18	9
IM	34	56	71	68	47	20	9

*30-minute infusion

PLASMA CONCENTRATIONS AFTER 2.0 GRAM IV* or IM DOSE

Mean Plasma Concentration (μg/mL)

	Time After Injection						
Route	5 min	10 min	1 h	3 h	5 h	9 h	12 h
IV	237	223	135	74	48	22	12**
IM	–	20	75	91	69	33	19

*Injected over 3 minutes

**Concentrations estimated from regression line

The plasma elimination half-life of cefotetan is 3 to 4.6 hours after either intravenous or intramuscular administration.

Repeated administration of CEFOTAN™ (cefotetan disodium) does not result in accumulation of the drug in normal subjects.

Cefotetan is 88% plasma protein bound.

No active metabolites of cefotetan have been detected; however, small amounts (less than 7%) of cefotetan in plasma and urine may be converted to its tautomer, which has antimicrobial activity similar to the parent drug.

In normal patients, from 51% to 81% of an administered dose of CEFOTAN™ is excreted unchanged by the kidneys over a 24 hour period, which results in high and prolonged urinary concentrations. Following intravenous doses of 1 gram and 2 grams, urinary concentrations are highest during the first hour and reach concentrations of approximately 1700 and 3500 μg/mL respectively.

In patients with reduced renal function, the plasma half-life of cefotetan is prolonged.

Therapeutic levels of cefotetan are achieved in many body tissues and fluids including:

skin	endometrium	ureter	bile
muscle	cervix	bladder	peritoneal fluid
fat	ovary	maxillary sinus mucosa	umbilical cord serum
myometrium	kidney	tonsil	amniotic fluid

MICROBIOLOGY

The bactericidal action of cefotetan results from inhibition of cell wall synthesis. Cefotetan has in vitro activity against a wide range of gram positive and gram negative organisms. The methoxy group in the 7-alpha position provides cefotetan with a high degree of stability in the presence of beta-lactamases, both penicillinases and cephalosporinases of gram-negative bacteria. Cefotetan is usually active against the following organisms in vitro and in clinical infections.

GRAM-NEGATIVE:

Escherichia coli

Klebsiella species (including *K pneumoniae*)

Enterobacter aerogenes

Enterobacter agglomerans

Enterobacter cloacae

Proteus mirabilis

Proteus vulgaris

Morganella morganii (formerly *Proteus morganii*)

Providencia rettgeri (formerly *Proteus rettgeri*)

Providencia stuartii

Haemophilus influenzae (including ampicillin-resistant strains)

Neisseria gonorrhoeae

NOTE: Approximately one-half of the strains of *Enterobacter* species, and *Citrobacter* species are resistant to cefotetan. Most strains of *Pseudomonas aeruginosa* and *Acinetobacter* species are resistant to cefotetan.

GRAM-POSITIVE:

Staphylococcus aureus (including penicillinase- and nonpenicillinase-producing strains)

Staphylococcus epidermidis

Streptococcus pyogenes (group A beta-hemolytic streptococci)

Streptococcus agalactiae (group B beta-hemolytic streptococci)

Streptococcus pneumoniae (formerly *Diplococcus pneumoniae*)

NOTE: Methicillin-resistant staphylococci are resistant to cephalosporins. Some strains of *Staphylococcus epidermidis* and most strains of enterococci, e.g., *S faecalis*, are resistant to cefotetan.

ANAEROBES:

Bacteroides fragilis subsp *fragilis*

Bacteroides bivius

Bacteroides disiens

Bacteroides melaninogenicus

Bacteroides vulgatus

Fusobacterium species

Gram-positive bacilli (including *Clostridium* species)

Peptococcus and *Peptostreptococcus* species

NOTE: Most strains of *B distasonis*, *B ovatus*, and *B thetaiotaomicron* are resistant to cefotetan.

Cefotetan also demonstrates in vitro activity against the following microorganisms, although its clinical significance is unknown: *Citrobacter* species (including *C diversus* and *C freundii*), *Serratia* species (including *S marcescens*), *Salmonella* species, *Shigella* species, *Yersinia enterocolitica*, *Clostridium difficile*, *B asaccharolyticus*, *B oralis*, *B splanchnicus*, *Veillonella* species, and *Propionibacterium* species.

NOTE: Many strains of *C difficile* are resistant.

SUSCEPTIBILITY TESTS

Diffusion Technique: Quantitative methods that require measurement of zone diameters give the most precise estimate of antibiotic susceptibility. One such procedure[1] has been recommended for use with disks to test susceptibility to cefotetan. Organisms should be tested with the cefotetan 30 μg disk since cefotetan has been shown to be active in vitro against organisms which were found to be resistant to other beta-lactam antibiotics.

Reports from the laboratory with results of standardized single-disk susceptibility tests with a 30 μg cefotetan disk should be interpreted according to the following criteria:

Susceptible organisms produce zone sizes of 16 mm or greater, indicating that the tested organism is likely to respond to therapy.

Moderately susceptible organisms produce zones of 13 to 15 mm indicating the test organism would be susceptible if high dosage is used or if the infection is confined to tissues and fluids (e.g., urine) in which high antibiotic levels are attained.

Resistant organisms produce zones of 12 mm or less, indicating that other therapy should be selected.

Standardized procedures require the use of laboratory control organisms. The 30 μg cefotetan disk should give zone diameters between 17 and 22 mm for *S aureus* ATCC 25923. For *E coli* ATCC 25922, the zone diameters should be between 29 and 34 mm.

Dilution Techniques: Broth or agar dilution methods, e.g., ICS agar dilution[2] or an equivalent method such as that recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[3], may be used to determine the minimal inhibitory concentration (MIC) of cefotetan.

Organisms inhibited by cefotetan at 16 μg/mL or less are considered susceptible. Organisms with MIC values 64 μg/mL or more are considered resistant to cefotetan.

As with standard diffusion methods, dilution procedures require the use of laboratory control organisms. Standard cefotetan powder should give MIC values in the range of 4 μg/mL and 16 μg/mL for *S aureus* ATCC 25923. For *E coli* ATCC 25922, the MIC range should be between 0.06 μg/mL and 0.25 μg/mL.

INDICATIONS AND USAGE

TREATMENT

CEFOTAN™ (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus spp* (which may include the organisms now called *Proteus vulgaris*, *Providencia rettgeri*, and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and non-penicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli*.

Skin and Skin Structure Infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli*.

Gynecologic Infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*, *Streptococcus* species (excluding enterococci), *E coli*, *Proteus mirabilis*, *Neisseria gonorrhoeae*, *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*), *Fusobacterium* species*, and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).

Intra-abdominal Infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *B thetaiotaomicron*).

Bone and Joint Infections caused by *Staphylococcus aureus**.

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN™ concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN™ and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN™ was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN™ is used concomitantly with an aminoglycoside.

PROPHYLAXIS

The preoperative administration of CEFOTAN™ may reduce the incidence of certain post-operative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (e.g., cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN™ should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN™ should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN™ (cefotetan disodium) is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

(CONTINUED ON REVERSE SIDE)

WARNINGS

Before therapy with CEFOTAN™ is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN™ occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C. difficile*. Other causes should also be considered.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN™ may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN™ may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment, or poor nutritional state. Prothrombin times should be monitored in patients at risk and exogenous Vitamin K administered as indicated.

CEFOTAN™ should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc) is ingested within 72 hours after CEFOTAN™ administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN™.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN™ was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN™ is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels of Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approx. 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and pre-puberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes. Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent, at 120 mg/kg/day (approx. 2-4 times the usual human dose), only 1 of 10 treated animals was affected, and the degree of degeneration was mild. Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Usage in Pregnancy: Pregnancy Category B. Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies the following adverse effects were considered related to CEFOTAN™ (cefotetan disodium) therapy.

Gastrointestinal symptoms occurred in 1.5 percent of patients, the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4 percent of patients and included eosinophilia (1 in 200), positive direct Coomb's test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2 percent of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2 percent of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than one percent of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

DOSAGE AND ADMINISTRATION

TREATMENT

The usual adult dosage is 1 or 2 grams of CEFOTAN™ administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN™

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS

To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN™ (cefotetan disodium) administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION

When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dose*	Every 12 hours
10-30	Usual Recommended Dose*	Every 24 hours
<10	Usual Recommended Dose*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males: } \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

Females: 0.9 X value for males

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

PREPARATION OF SOLUTION

For Intravenous Use: Reconstitute with Sterile Water for Injection. Shake to dissolve and let stand until clear.

Vial Size	Amount of Diluent To Be Added (mL)	Approximate Withdrawable Vol (mL)	Approximate Average Concentration (mg/mL)
1 gram	10	10.4	97.2
2 gram	10-20	11.0-21.0	179.8-96.4

Infusion bottles (100 mL) may be reconstituted with 50 to 100 mL of 5% Dextrose Solution or 0.9% Sodium Chloride Solution.

For Intramuscular Use: Reconstitute with Sterile Water for Injection; Bacteriostatic Water for Injection, Normal Saline, USP, 0.5% Lidocaine HCl; or 1.0% Lidocaine HCl. Shake to dissolve and let stand until clear.

Vial Size	Amount of Diluent To Be Added (mL)	Approximate Withdrawable Vol (mL)	Approximate Average Concentration (mg/mL)
1 gram	2	2.4	375.0
2 gram	3	3.9	471.5

INTRAVENOUS ADMINISTRATION

The intravenous route is preferable for patients with bacteremia, bacterial septicemia, or other severe or life-threatening infections, or for patients who may be poor risks because of lowered resistance resulting from such debilitating conditions as malnutrition, trauma, surgery, diabetes, heart failure, or malignancy, particularly if shock is present or impending.

For intermittent intravenous administration, a solution containing 1 gram or 2 grams of CEFOTAN™ in Sterile Water for Injection can be injected over a period of three to five minutes. Using an infusion system, the solution may also be given over a longer period of time through the tubing system by which the patient may be receiving other intravenous solutions. Butterfly® or scalp vein-type needles are preferred for this type of infusion. However, during infusion of the solution containing CEFOTAN™, it is advisable to discontinue temporarily the administration of other solutions at the same site.

NOTE: Solutions of CEFOTAN™ must not be admixed with solutions containing aminoglycosides. If CEFOTAN™ and aminoglycosides are to be administered to the same patient, they must be administered separately and not as a mixed injection.

INTRAMUSCULAR ADMINISTRATION

As with all intramuscular preparations, CEFOTAN™ should be injected well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel.

COMPATABILITY AND STABILITY

CEFOTAN™ reconstituted as described above (Preparation of Solution) maintains satisfactory potency for 24 hours at room temperature (25°C), for 96 hours under refrigeration (5°C), and for at least 1 week in the frozen state. After reconstitution and subsequent storage in disposable glass or plastic syringes, CEFOTAN™ is stable for 24 hours at room temperature and 96 hours under refrigeration.

Frozen samples should be thawed at room temperature before use. After the periods mentioned above, any unused solutions or frozen materials should be discarded. Do not refreeze.

NOTE: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

HOW SUPPLIED

CEFOTAN™ (cefotetan disodium) is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-[illegible])

2 g in 20 mL vial (NDC 0038-0377-20)

1 g in 100 mL vial (NDC 0038-0376-11)

2 g in 100 mL vial (NDC 0038-0377-21)

1 Am J Clin Pathol 1966;45:493
Federal Register 1974;39:19182-19184

2 Enceon HM, Sherris JC. Acta Pathol Microbiol Immunol Scand (B) 1971, (suppl No. 217)

3 Thornsberry C, et al. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 1983, Vol 3, No. 2

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WILMINGTON, DELAWARE 19897

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ATTACHMENT C

United States Patent [19]

Iwanami et al.

[11] 4,263,432

[45] Apr. 21, 1981

[54] 7α-METHOXY-7β-(1,3-DITHIETANE-2-CARBOXAMIDO)CEPHALOSPORANIC ACID DERIVATIVES

[75] Inventors: Masaru Iwanami, Yokohama; Tetsuya Maeda, Urawa; Yoshinobu Nagano, Niiza; Masaharu Fujimoto, Tokyo; Noriaki Nagano, Ageo; Atsuki Yamazaki, Ichikawa, all of Japan

[73] Assignee: Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan

[21] Appl. No.: 913,500

[22] Filed: Jun. 7, 1978

[30] Foreign Application Priority Data

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Jul. 28, 1977 [JP] Japan 52-90772
Feb. 2, 1978 [JP] Japan 53-10772

[51] Int. Cl.³ C07D 501/18

[52] U.S. Cl. 544/21; 424/246; 544/27

[58] Field of Search 544/16, 21, 27, 26; 424/246

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Primary Examiner—Nicholas S. Rizzo

Attorney, Agent, or Firm—Burgess, Ryan and Wayne

[57] ABSTRACT

The invention relates to novel 7α-methoxycephalosporin derivatives having at the 7β-position an amido group acylated by a 1,3-dithietane carboxylic acid residue and to 7β-isothiazolylthioacetamido-7α-methoxycephalosporins having antibacterial activity by themselves and, as the case may be, capable of being converted into the aforesaid 7α-methoxycaphalosporin derivatives. The compounds of this invention exhibit excellent antibacterial activity and are particularly effective against gram negative bacteria.

9 Claims, No Drawings

7α-METHOXY-7β-(1,3-DITHIETANE-2-CARBOXAMIDO)CEPHALOSPORANIC ACID DERIVATIVES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a novel series of cephalosporin compounds having antibacterial activity and to intermediates useful for preparing them. To the best of our knowledges, the compounds of this invention disclosed herein are novel.

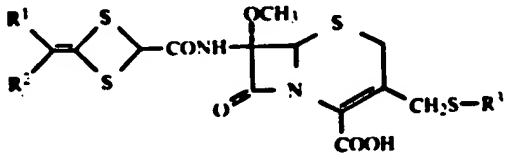
2. Description of the Prior Art

In the past several decades various antibiotics have been investigated and used for the treatment of various infectious diseases of animals including men but since resistant bacteria appear in many cases, there are some infectious diseases which have not been treated by known antibiotics. Moreover, new antibiotics are constantly being sought in order to supplement and expand the physicians' armamentarium, particularly for the treatment of infections involving pathogens which have become resistant to the chemotherapeutic agents now in use.

Various cephalosporins have been known and a number of disclosures such as German Offenlegungsschrift No. 2,356,388 disclose a variety of cephalosporins or heterocyclic acyl groups very broadly but none of them specify the compounds of this invention.

SUMMARY OF THE INVENTION

According to this invention, there are provided the novel cepholosporins represented by general formula I



I

wherein R^1 represents a carboxyl group or a functional derivative residue thereof; R^2 represents a hydrogen atom, a lower alkyl group, a lower alkoxy group, a lower alkanoyl group, $R^4S(O)_n$ group (wherein R^4 represents a lower alkyl group and n represents 0, 1, or 2), an aryl group, an aroyl group, a carboxyl group, a functional derivative group of a carboxyl group, a lower alkenyl group, a sulfamoyl group, or a heterocyclic residue; and R^3 represents a lower alkyl-substituted tetrazolyl group or a lower alkylsubstituted thiadiazolyl group, and the pharmaceutically acceptable salts thereof.

The cephalosporin compounds of this invention have excellent antibacterial activity, particularly against gram negative bacteria.

The invention also provides various processes for preparing the aforesaid cephalosporin compounds of general formula I.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Now, the functional derivative residue of carboxyl group represented by R^1 or R^2 of general formula I means, for example, a carboxylic acid lower alkyl ester residue, a carboxylic acid aralkyl ester residue, a carbamoyl group, a carbazoyl group (NH_2NHCO—), a

cyano group, etc. Also, the lower alkyl group in the general formula is a straight chain or branched alkyl group having 1–4 carbon atoms, such as methyl group, ethyl group, isopropyl group, n-butyl group, tert-butyl group, etc.

Examples of the aryl group are phenyl group, naphthyl group, etc. Examples of the aroyl group are benzoyl group, naphthoyl group, etc.

R^3 of general formula I represents, as described above, a lower alkyl-substituted tetrazolyl group or a lower alkylsubstituted thiadiazolyl group, and examples of the tetrazolyl group are a 1H-tetrazol-5-yl group, 2H-tetrazol-5-yl group, etc., and examples of the thiadiazolyl group are a 1,3,4-thiadiazolyl group, a 1,2,5-thiadiazolyl group, a 1,2,4-thiadiazolyl group, etc.

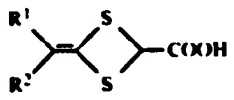
Furthermore, the groups and residues represented by R^1, R^2, R^3 and R^4 of general formula I may have been substituted when they can have substituents. For example, examples of the substituted groups or residues are an N-monoalkylcarbamoyl group, an N-dialkylcarbamoyl group, and an alkoxycarbonylamino group for R^1; a hydroxyalkyl group, a carboxyalkyl group, an alkoxyalkyl group, an arylalkyl group, a hydroxyphenyl group, and an alkoxyphenyl group for R^2; and an alkylthio-substituted thiadiazolyl group, for R^3, etc.

The compounds of this invention belong to 7-methoxycephalosporin derivatives as shown by general formula I and the most specific feature of the compounds is that the acyl group at the 7β-position originates in 4-substituted methylene-1,3-dithietane-2-carboxylic acid. The acylation by 4-membered ring carboxylic acid has not hitherto been known in the field of cephalosporin chemistry and, in particular, the 1,3-dithietanecarboxylic acid itself used in one of the processes for preparing the compounds of this invention shown below is a novel compound which has not been disclosed in any literatires.

The objective compounds of this invention are prepared by the following various processes:

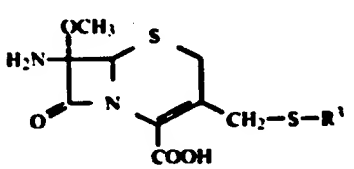
Process 1

In the process, the compounds of this invention shown by general formula I are prepared by reacting 4-substituted methylene-1,3-dithietanecarboxylic acid represented by general formula II



II

wherein R^1 and R^2 have the same significance as in general formula I and 7β-amino-7α-methoxy-3-heterocyclic thiomethyl-Δ^3-cephem-4-carboxylic acid represented by general formula III



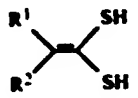
III

wherein R^3 has the same significance as in general formula I.

In the reaction of the compounds of general formula II and the compounds of general formula III, the com-

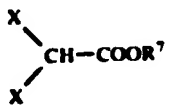
pounds may be caused to react directly with each other in the presence of a condensing agent such as N,N'-dicyclohexylcarbodiimide, etc., but it is suitable to use the compound of formula II after introducing known protective groups to R^1 and R^2 according to the properties of R^1 and R^2 and also the compounds of formula III after introducing a known protective group to the carboxyl group at the 4-position. For example, when R^1 and/or R^2 of the compound of formula II is a carboxyl group, the carboxyl group of the compound and also the carboxyl group at the 4-position of the compounds of formula III are protected beforehand by a triphenylmethyl group, a tert-butyl group, a benzhydryl group, etc., and further the carboxylic acid at the 2-position of the compound of formula II or the amino group at the 7β-position of the compound of formula III are converted into the reactive derivatives prior to performing the reaction. Preferred examples of the reactive derivative of the carboxylic acid are an acid halide, a mixed acid anhydride, an active ester, an active amide, an acid anhydride, an acid azide, etc.

The compounds of general formula II are novel compounds and they are obtained by reacting 2,2'-substituted ethylene-1,1-dithiol represented by general formula IV



IV

wherein R^1 and R^2 have the same significance as in general formula I and dihalogenoacetic acid or the lower alkyl ester thereof represented by general formula V



V

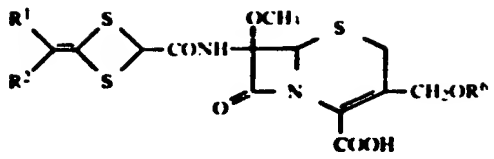
wherein X represents a halogen atom and R^7 represents a hydrogen atom or a lower alkyl group and then, when the compound of formula V is the lower alkyl ester, releasing the alkyl or converting it to a reactive derivative.

The reaction of the compounds of formula II and the compounds of formula III or the reactive derivative thereof is usually carried out in an inert solvent under heating or cooling but in order to avoid the epimerization of the methoxy group at the 7α-position during the reaction, it is preferred to perform the reaction at low temperature, particularly at temperatures below −20° C.

The compound thus formed can be converted into the compounds of formula I by removing the protective group or groups in an ordinary manner.

Process 2

In the process, the compounds of general formula I are prepared by reacting 3-acetoxymethyl- or 3-carbamoyloxymethyl-7β-(4-substituted-1,3-dithietanecarboxamido)-7α-methoxy-Δ^3-cephem-4-carboxylic acid represented by general formula VI



VI

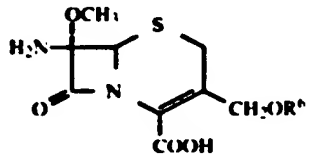
wherein R^1 and R^2 have the same significance as in general formula I and R^6 represents an acetyl group or a carbamoyl group and the heterocyclic thiol represented by general formula VII

$$R^3-SH \qquad \text{VII}$$

wherein R^3 has the same significance as in general formula I or the alkali metal substitute thereof at the hydrogen atom of the mercapto group.

The reaction is performed at room temperature or under heating usually in an inert solvent. Examples of the inert solvent are acetone, dimethylformamide, methanol, ethanol, water, and a phosphate buffer and, if necessary, they are used as a mixture of them. When a compound of general formula VII is used in the free state, it is preferred to perform the reaction in the presence of a base such as an alkali metal hydroxide, an alkali metal carbonate, an alkali metal hydrogencarbonate, trialkylamine, pyridine, dimethylaniline, etc. After the reaction is over, the compound of formula I formed is isolated by acidifying the reaction mixture and recovering the precipitates thus formed or by subjecting the reaction mixture to a solvent extraction.

In addition, a compound of formula VI used in the process can be obtained by reacting a compound of general formula II used in Process 1 and 7β-amino-7α-methoxycephalosporanic acid (R^6 in the formula is acetyl group) or 7β-amino-3-carbamoyloxymethyl-7α-methoxy-Δ^3-cephem-4-carboxylic acid (R^6 in the formula is $-CONH_2$) represented by general formula VIII

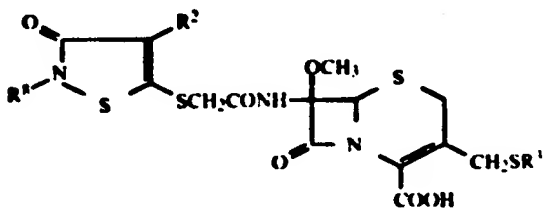


VIII

wherein R^6 has the same significance as in general formula VI under similar reaction condition as in Process 1.

Process 3

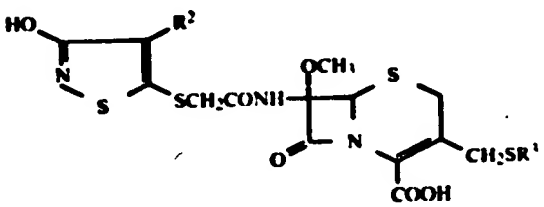
A compound of this invention shown by general formula I is also obtained by treating the 7α-methoxy-3-heterocyclic thiomethylcephalosporin derivative represented by general formula IX



IX

wherein R^2 and R^3 have the same significance as in general formula I and R^8 represents a hydrogen atom or a substituted or unsubstituted alkyl group under a basic condition.

In addition, when R^8 of general formula IX is hydrogen, the derivative of the formula includes the tautomer of the following formula



IX'

wherein R^2 and R^3 are the same as above.

Proper bases used in this process are weak bases such as sodium hydrogencarbonate, potassium hydrogencarbonate, sodium carbonate, etc. The reaction is usually performed in a solvent at room temperature or under cooling. Any solvents which do not affect the reaction may be used but preferably water or an organic solvent which are miscible with water, such as methanol, acetone, tetrahydrofuran, dimethylformamide, etc., are used singly or in a combination thereof. The isolation and purification of the product from the reaction mixture are performed by a conventional manner such as extraction with organic solvent, crystallization, column chromatography, etc.

The compounds of general formula IX used in the process exhibit an excellent antibacterial activity per se and are also useful as intermediates for the preparation compounds of formula I. Therefore, another object of this invention is to provide the intermediate compounds which have excellent antibacterial activity and are useful for the production of the compounds of formula I and also to provide a process of preparing the intermediate compounds.

The compounds of general formula IX are prepared by, for example, the following processes:

Process A

The compounds of general formula III may be reacted with a corresponding isothiazolylthioacetic acid or the reactive derivative thereof according to Process 1 of this invention. That is, the reaction is usually performed in an inert solvent such as, preferably, acetone, chloroform, methylene chloride, ethylene chloride, tetrahydrofuran, dimethylformamide, acetonitrile, ethyl acetate, ethyl formate, etc. These solvents may be used singly or in a combination thereof, or, if the solvent is water-soluble, the solvent can be used as a mixture with water if no hindrance occurs in the reaction.

The preferred examples of the reactive derivative at the terminal carboxyl group of isothiazolylthioacetic acid are an acid halide, a mixed acid anhydride, an active ester, an active amide, an acid anhydride, an acid azide, etc. When the terminal carboxyl group is a free radical, it is suitable to use a condensing agent such as N,N'-dicyclohexylcarbodiimide, N,N'-diethylcarbodiimide, etc. Also, when R^2 of the isothiazolylthioacetic acid is a reactive group which may hinder the reaction, such as carboxyl group, hydroxymethyl group, etc., it is preferred to use the isothiazolylthioacetic acid in the reaction after introducing a conventional protective group to the reactive group. In this case, it is preferred to release the protective group after obtaining the com-

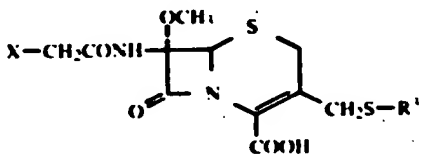
pound of formula IX, or after converting the compound of formula IX to the compound of formula I.

Process B

The compounds of general formula IX can be also obtained by reacting a compound shown by general formula VIII and the corresponding isothiazolylthioacetic acid or the reactive derivative thereof and then reacting the product with a compound shown by general formula VII, according to Process 2.

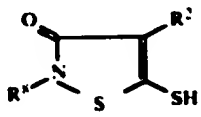
Process C

The compounds of general formula IX are further obtained by reacting the known 7α-amino-7β-haloacetamido-3-heterocyclic thiomethyl-Δ^3-cephem-4-carboxylic acid shown by general formula X



X

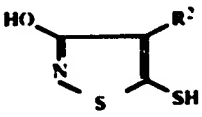
wherein R^1 has the same significance as above and X represents a halogen atom and a compound shown by general formula XI



XI

wherein R^2 and R^3 have the same significance as above and R^8 represents a hydrogen atom or a substituted or unsubstituted alkyl group under a basic condition.

In addition, when R^8 of general formula XI is a hydrogen atom, the compounds of formula XI include the tautomer thereof shown by the following formula



XI'

wherein R^2 has the same significance as above.

The reaction is carried out usually in a solvent at room temperature or under cooling. Any solvents which do not take part in the reaction may be used without restriction but ordinary, water, methanol, acetone, tetrahydrofuran, dimethylformamide or a mixture thereof is used as the solvent. The compounds of formula XI may be usually used as the alkali metal salt thereof at the mercapto group but when a compound of formula XI is used as it is, the reaction is carried out in the presence of an aliphatic, aromatic or heterocyclic base such as triethylamine, N,N-dimethylaniline, N-ethyl-morpholine, pyridine, collidine, 2,6-lutidine, etc., or an alkali metal carbonate or alkali metal hydrogencarbonate such as sodium carbonate, potassium carbonate, sodium hydrogencarbonate, potassium hydrogencarbonate, etc.

The compounds of general formula IX thus obtained are novel compounds. The compounds having an isothiazolylacetamide group at the 7-position have hitherto been known in the chemistry of cephalosporins as

described in, for example, U.S. Pat. No. 3,464,999, but the compounds of the specific structure having an isothiazolylthioacetamide group which can be converted into a 1,3-dithietanecarboxamide group have not yet been known.

The compounds obtained by the invention exhibit excellent antibacterial activity, particularly against gram negative bacteria as shown below.

TABLE

Example No.	*Escherichia Coli* NIHJ	*Klebsiella pneumoniae* ATCC 10031	*Proteus vulgaris* OXK US	*Proteus morganii* Kono	*Serratia marcescens*
	(M.I.C.) (γ/ml)				
1	0.2	0.2	1.56	1.56	3.13
2	0.78	1.56	3.13	12.5	6.25
4	0.78	0.78	3.13	6.25	6.25
5	0.09	0.09	0.78	1.56	0.78
6	0.09	0.09	0.39	0.78	0.39
7	0.78	0.39	0.78	6.25	6.25
8	0.09	0.09	0.39	0.39	0.39
9	0.19		0.39	0.78	0.78
11	0.19	0.19	1.56	0.39	0.39
12	0.78	0.39	0.78		
14	0.19	0.19	1.56	1.56	0.78
15	0.19	0.19	1.56	3.13	0.78
16	0.39	0.39	0.78	6.25	6.25
17	0.39	0.39	0.78	0.39	12.5
18	0.78	0.78	3.13		
19	0.19	0.09	0.78	0.39	1.56
22	0.78	0.39	0.78	3.13	3.13
23	1.56	1.56	1.56	6.25	6.25
24	0.39	0.39	0.78	3.13	0.78
25	0.19	0.09	0.78	0.39	0.39
26	0.78	0.39	1.56	1.56	0.78
29	0.78	0.78	1.56	6.25	1.56
30	0.09	0.20	0.78		
32	≦0.20	0.20	0.78	0.78	0.39

The compounds of formula I obtained in this invention can be easily converted into the pharmaceutically acceptable, non-toxic or effective salts thereof. These salts includes the alkali metal salts such as sodium or potassium salt or salts (for example, using sodium or potassium 2-ethyl hexanoate), ammonium salt or salts, and organic amine salt or salts such as those with procaine or ethanolamine which can be prepared by one skilled in the art according to known manners.

Moreover, the pharmaceutical compositions having an actibacterial activity comprising a pharmaceutical carrier and an active but non-toxic amount of the compound of formula I as well as the methods of combatting bacterial infections by administering such a pharmaceutical composition to an infected animal or human host in an non-toxic amount sufficient to combat such infections are also the objects of this invention.

The compounds of this invention may be adminstered orally, rectally, or by injection such as subcutaneously, intramuscularly, or intravenously.

The injection of suitably prepared sterile solutions or suspensions containing an effective but non-toxic amount of the cephalosporin compound of this invention is the preferred route of administration.

The doses of the cephalosporin compound of this invention are usually 250–3000 mg. per day for an adult and can be variously changed according the condition of disease, the age, weight, and the state of the patient.

Then, the invention will further be described in more detail by referring to the following examples.

EXAMPLE 1

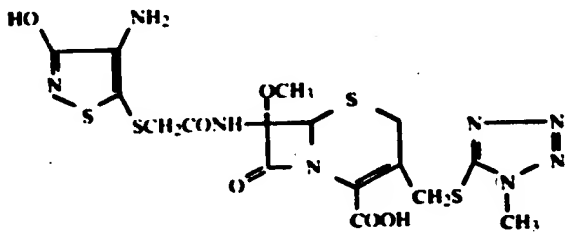
In 10 ml. of liquid ammonia was suspended 270 mg. of 4-carboxy-5-ethylthio-3-hydroxyisothiazole. After cooling the suspension to −50° C. and adding thereto 100 mg. of metallic sodium, the mixture was stirred for 30 minutes at temperatures of from −50° C. to −33° C.

Liquid ammonia was distilled off from the reaction mixture, the residue obtained was dissolved in 20 ml. of methanol, then 10 ml. of a methanol solution of 600 mg. of 7β-bromoacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid was added dropwise to the solution under ice-cooling, and after stirring the mixture for 30 minutes under ice-cooling, the mixture was further stirred for 30 minutes at room temperature. After the reaction was over, the reaction mixture was adjusted to pH 4 with 4 normal hydrochloric acid and then the reaction solvent was distilled off under reduced pressure.

To the residue formed was added water and after adjusting the mixture to pH 1 with 4 normal hydrochloric acid, the product was extracted with 50 ml. of a mixture of butanol and ethyl acetate of 1:1 by volume ratio. The organic layer formed was washed twice each time with water, then once with a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure. To the residue was added 30 ml. of ether and the precipitates formed were recovered by filtration, washed three times each time with 20 ml. of ether, and dried under reduced pressure to provide 560 mg. of the powder of 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.41 (3H), 3.58 (2H), 3.93 (3H), 3.99 (2H), 4.28 (2H), 5.10 (1H).

EXAMPLE 2

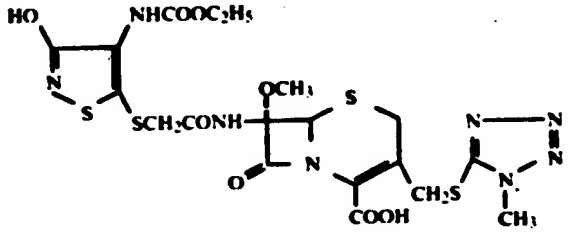


After cooling 40 ml. of liquid ammonia to −70° C., 183 mg. of 4-amino-5-ethylthio-3-hydroxyisothiazole, 55 mg. of sodium was added to the liquid ammonia. The mixture was stirred for 10 minutes at the same temperature and then liquid ammonia was distilled off. To the residue was added 15 ml. of methanol followed by cooling to 2° C. Then 15 ml. of a methanol solution of 300 mg. of 7β-bromoacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid was added dropwise to the mixture over a period of 30 seconds followed by stirring for 10 minutes at the same temperature. The solvent was distilled off under reduced pressure and after adding 15 ml. of water to the residue, the mixture was adjusted to pH 2.5 by adding 5% hydrochloric acid. The precipitates formed were extracted with 100 ml. of a mixture of n-butanol and ethyl acetate of 1:1 volume ratio and the extract was washed with water and then with a saturated aqueous

sodium chloride solution, and dried over anhydrous magnesium sulfate. The solvent was distilled off and the residue formed was subjected to a silica gel column chromatography to provide 180 mg. of 7β-(4-amino-3-hydroxyisothiazol-5-yl)thioacetamido-7β-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid using a mixture of chloroform, methanol, and formic acid of 8:2:0.2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.36 (3H), 3.54 (2H), 3.58 (2H), 3.94 (2H), 4.30 (2H), 5.09 (1H).

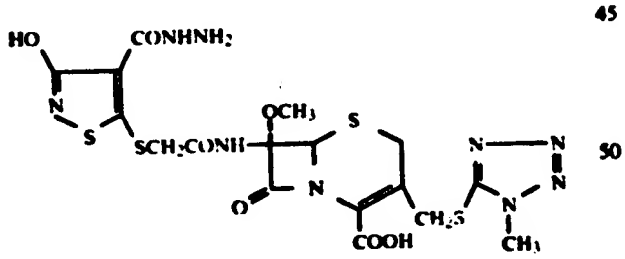
EXAMPLE 3



By following the same procedure as in Example 2, 50 mg. of 7β-(4-ethoxycarbonylamino-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid was obtained from 300 mg. of 4-ethoxycarbonylamino-5-ethylthio-3-hydroxyisothiazole and 300 mg. of 7β-bromoacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 1.14 (3H), 3.38 (3H), 3.60 (2H), 3.81 (2H), 3.92 (3H), 4.06 (2H), 4.29 (2H), 5.11 (1H).

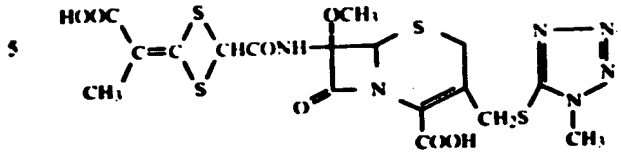
EXAMPLE 4



By following the same procedure as in Example 2, 100 mg. of 7β-(4-carbazolyl-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid was obtained from 220 mg. of 4-carbazoyl-5-ethylthio-3-hydroxyisothiazole and 400 mg. of 7β-bromoacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.39 (3H), 3.63 (2H), 3.90 (2H), 3.93 (3H), 4.30 (2H), 5.13 (1H).

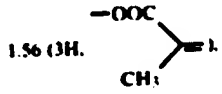
EXAMPLE 5



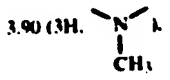
(a). In 15 ml. of methylene chloride were dissolved 0.340 g. of 4-[1-(tert-butoxycarbonyl)ethylidene]-1,3-dithietane-2-carboxylic acid and 0.206 g. of pyridine. Then, while stirring the solution in an ice-water bath, 0.284 g. of phosphorus pentachloride was added to the solution. The reaction was carried out for one hour at temperature below 10° C. and then after cooling the reaction mixture to −50° C., a solution of 0.690 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester in 10 ml. of methylene chloride was added dropwise to the solution and then 1.6 ml. of pyridine was added dropwise and the mixture was reacted for one hour at temperatures of from −30° C. to −40° C.

After the reaction was over, 10 ml. of 5 normal hydrochloric acid was added dropwise to the reaction mixture below 0° C. and the product was extracted with methylene chloride. The extract was washed with a saturated aqueous sodium chloride solution, dried over anhydrous calcium chloride, and then methylene chloride was distilled off to provide 1.1 g. of a residue. The residue was subjected to a silica gel column chromatography and then 0.490 g. (yield 47%) of caramel-like 7β-{4-[1-(tert-butoxycarbonyl)ethylidene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester was obtained using a mixture of ethyl acetate and n-hexane of 1:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 1.44 (9 H, tert-butyl),



3.49 (3H—OCH_3),

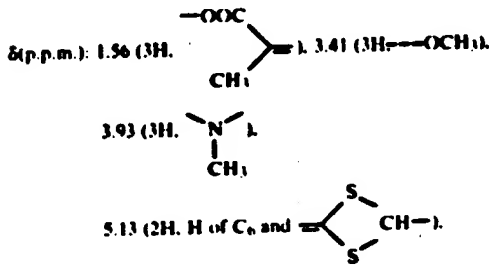


6.92 (1H, —CH(C_6H_5)$_2$),
9.68 (1H, —CONH—).

(b). In 25 ml. of anisole was dissolved 0.44 g. of the product obtained in step (a) and while cooling the solution below 5° C. with ice-water, 7.5 ml. of trifluoroacetic acid was added dropwise to the solution. The reaction was performed for one hour at 5°-10° C., anisole and excess trifluoroacetic acid were distilled off under reduced pressure, and the residue was powdered by

adding thereto ether. After recovering the powder by filtration, the powder was washed well with ether to provide 0.271 g. (yield 86.7%) of the light yellow powder of 7β-[4-(1-carboxyethylidene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO)



9.57 (1H, —CONH—)

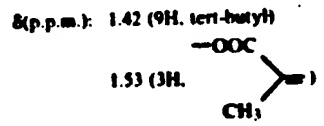
Infrared spectrum (KBr) (cm^{-1}) 1870 (lactam).

REFERENCE EXAMPLE 1

In a 100 ml. three-necked flask were placed 40 ml. of dimethoxyethane and 10 ml. of tetrahydrofuran both were deoxygenated by distillation. While cooling the mixture below −70° C. by a dry ice acetone bath in nitrogen stream, 1 ml. of n-isopropylcyclohexylamine and 3.43 ml. of a 15% n-butyl lithium n-hexane solution were added to the mixture. Then, after adding thereto 0.65 g. of tert-butyl propionate, the reaction was carried out for about 30 minutes at temperature below −70° C. with stirring. To the reaction mixture was added dropwise 0.332 ml. of carbon disulfide at temperatures of from −75° C. to −73° C. over a period of about 30 minutes. The reaction was further carried out for 10 minutes at temperature below −70° C. and then 3.4 ml. of a 15% n-butyl lithium n-hexane solution was added dropwise to the reaction mixture at temperature below −70° C. over a period of about 30 minutes. After carrying out the reaction for 15 minutes at temperature below −70° C., sodium diiodoacetate obtained beforehand by reacting 0.24 g. of 50% oily sodium hydride and 1.56 g. of diiodoacetic acid in 10 ml. of dimethoxyethane under ice-cooling, was added to the reaction mixture and the mixture was stirred overnight at room temperature.

The solvent was distilled off from the reaction mixture under reduced pressure and after adding cold ether to the residue and acidifying the residue with 1 normal hydrochloric acid, the product was extracted with ether. The ether extract was washed well with a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then ether was distilled off to provide 1.42 g. of a brown oily product. The product was subjected to silica gel column chromatography and 0.5 g. of oily 4-[1-(tert-butoxycarbonyl)-ethylidene]-1,3-dithietane-2-carboxylic acid was obtained using a mixture of chloroform, methanol, and formic acid of 95:5:2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra (D_6-DMSO)



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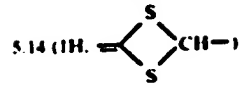
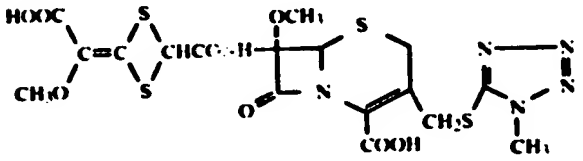


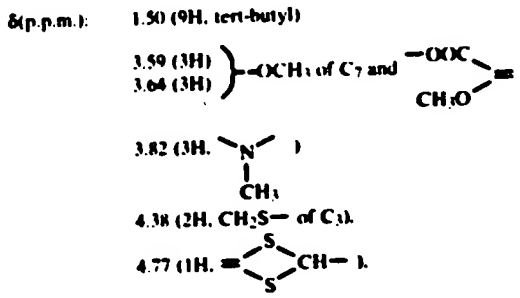
Infrared spectra (cm $^{-1}$): 2970 (tert-butyl), 2520–2650 (—COOH), 1640–1740 (—COO-tert-butyl, —COOH), 1360, 1250, and 840 (tert-butyl)

EXAMPLE 6



(a). In 5 ml. of tetrahydrofuran were dissolved 0.7 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester and 0.35 g. of 4-[(tert-butoxycarbonyl)(methoxy)methylene]-1,3-dithietane-2-carboxylic acid and after adding thereto 0.3 g. of N,N'-dicyclohexylcarbodiimide under ice-cooling, the mixture was stirred for two hours at room temperature. Insoluble materials were filtered off and the filtrate was concentrated under reduced pressure. The residue was subjected to a column chromatography and 0.36 g. of 7β-{4-[(tert-butoxycarbonyl)(methoxy)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester was obtained using a mixture of benzene and ethyl aceate of 85:15 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$)



δ(p.p.m.): 1.50 (9H, tert-butyl)

3.59 (3H), 3.64 (3H) } —OCH$_3$ of C$_7$ and —COC(=)CH$_3$O

3.82 (3H, N(CH$_3$)=)

4.38 (2H, CH$_2$S— of C$_3$)

4.77 (1H, =S(S)CH—)

5.07 (1H, H of C$_6$),

6.92 (1H, —CH(C$_6$H$_5$)$_2$),

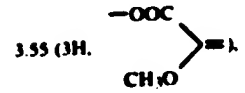
7.2–7.5 (10H, —CH(C$_6$H$_5$)$_2$)

(b). In 1.7 ml. of anisole was dissolved 0.23 g. of the product obtained in step (a) and while cooling the solution to temperature of from −5° C. to −10° C., 5.1 ml. of trifluoroacetic acid was added gradually followed by stirring for 30 minutes at 0°–8° C.

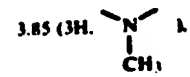
The reaction mixture was concentrated under reduced pressure, ether was added to the residue, and the faint brown powder formed was recovered by filtration. The powder was washed well with ether and dried under reduced pressure to provide 0.12 g. of 7β-{4-[(carboxy)(methoxy)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resoance spectra (D_6-DMSO)

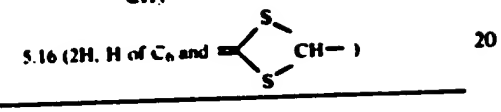
δ(p.p.m.): 3.42 (3H, —OCH_3 of C_7)



3.55 (3H,)



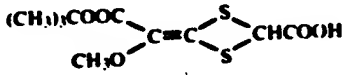
3.85 (3H,)



5.16 (2H, H of C_6 and)

9.59 (1H, —CONH—).

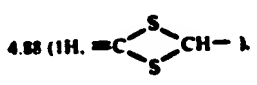
REFERENCE EXAMPLE 2



A mixture of 4.5 g. of tert-butyl methoxyacetate and 10 ml. of tetrahydrofuran was added to a lithium diisopropylamine solution prepared by adding 18.2 ml. of a 15% n-butyl lithium hexane solution to a mixture of 3 g. of diisopropylamine and 20 ml. of tetrahydrofuran at temperature of from −40° C. to −70° C. and then after adding thereto 0.9 ml. of carbon disulfide at temperature below −40° C., the resultant mixture was stirred for 20 minutes at the same temperature. Then, after adding to the resulting reaction mixture the lithium diisopropylamine solution of ½ of the aforesaid amount and carbon disulfide of ½ of the aforesaid amount at temperature of from −40° C. to −70° C. to cause reaction, the lithium diisopropylamine solution of ½ of the aforesaid amount and carbon disulfide of ½ of the aforesaid amount were further added to the mixture to cause reaction. Then 9 g. of sodium diiodoacetate was added to the reaction mixture followed by rising gradually the temperature and stirring for one hour at 0°-5° C. and further for one hour at room temperature. The reaction mixture obtained was concentrated under reduced pressure and after adding 20 ml. of 10% hydrochloric acid to the residue formed, the product was extracted with 100 ml. of benzene. The extract was washed with water and concentrated under reduced pressure. The residue formed was subjected to a silica gel column chromatography and 5.6 g. of 4-[(tert-butoxycarbonyl)(methoxy)-methylene]-1,3-dithietane-2-carboxylic acid was obtained using a mixture of chloroform and ethanol of 10:2-5 by volume ratio as the eluant.

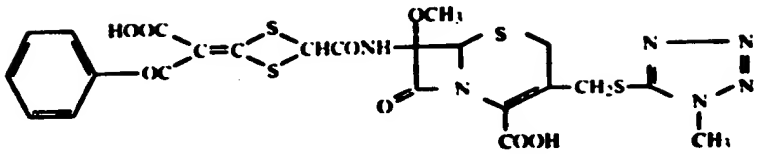
Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 1.52 (9H, $(CH_3)_3COOC$—), 3.67 (3H, CH_3O—),



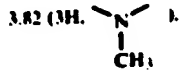
8.64 (1H, —COOH).

EXAMPLE 7

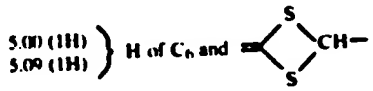


(a). By treating 0.8 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid benzhydryl ester and 0.8 g. of 4-[(benzoyl)(tert-butoxycarbonyl)methylene]-1,3-dithietane-2-carboxylic acid as in Example 6-(a), 0.35 g. of 7β-{4-[(benzoyl)(tert-butoxycarbonyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid benzhydryl ester was obtained.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.24 (9H, tert-butyl), 3.60 (3H, —OCH_3),



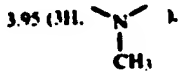
4.39 (2H, —CH_2S— of C_3),



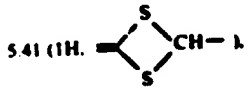
6.92 (1H, —$CH(C_6H_5)_2$),
7.2–7.6 (15H, H of aromatic ring),
7.77 (1H, —CONH—).

(b). By treating 0.23 g. of the product obtained in step (a) as in Example 6-(b), 0.13 g. of 7β-{4-[(benzoyl)(carboxy)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) (p.p.m.): 3.46 (3H, —OCH_3),

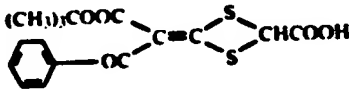


4.32 (2H, —CH_2— of C_2),
5.19 (1H, H of C_6),



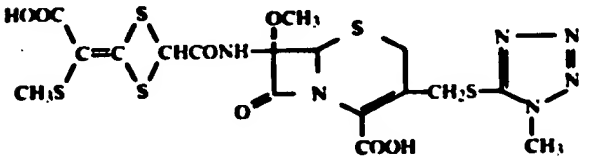
7.48 (5H, C_6H_5—OC—),
9.72 (1H, —CONH—).

REFERENCE EXAMPLE 3



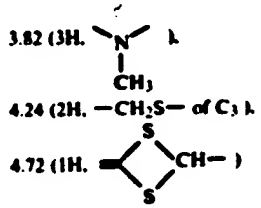
In a mixture of 2.2 g. of tert-butyl benzoylacetate and 20 ml. of tert-butanol was dissolved 0.24 g. of sodium hydride (50% in oil), and 0.6 ml. of carbon disulfide was added to the solution at 15°-20° C. followed by stirring for 40 minutes, 0.24 g. of sodium hydride (50% in oil) was then added to the mixture followed by stirring for one hour. To the reaction mixture obtained was added 1.52 g. of sodium dichloroacetate followed by stirring for 4 hours at room temperature. The reaction mixture was concentrated under reduced pressure and after adding 30 ml. of 1 normal hydrochloric acid to the residue formed, the product was extracted with 30 ml. of benzene. The extract was washed with water, dried, and concentrated under reduced pressure. By adding a mixture of benzene and n-hexane of 3:1 by volume ratio to the residue formed, 0.9 g. of the yellowish crystals of 4-[(benzoyl)(tert-butoxycarbonyl)methylene]-1,3-dithietane-2-carboxylic acid were obtained.

EXAMPLE 8



(a). In 10 ml. of anhydrous tetrahydrofuran were dissolved 0.3 g. of 4-[(tert-butoxycarbonyl)(methylthio)methylene]-1,3-dithietane-2-carboxylic acid, 0.2 g. of N,N'-dicyclohexylcarbodiimide, and 0.5 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester. Then the solution was stirred for one hour at room temperature. The solvent was distilled off under reduced pressure. The residue formed was subjected to a silica gel column chromatography and 0.3 g. of 7β-{4-[(tert-butoxycarbonyl)(methylthio)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester was obtained using a mixture of benzene and ethyl acetate of 9:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$)
δ(p.p.m.): 1.52 (9H, tert-butyl), 2.22 (3H, CH_3S—), 3.78 (5H, —OCH_3 and —CH_2— of C_2),

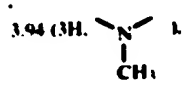


5.08 (1H, H of C_6),
6.92 (1H, —CH(C_6H_5)$_2$),
7.35 (10H, —CH(C_6H_5)$_2$),
7.80 (1H, —CONH—).

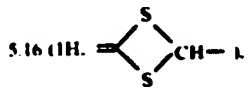
(b). In 1.5 ml. of anisole was dissolved 0.3 g. of the product obtained in step (a) and while stirring the solution at −5° C., 5 ml. of trifluoroacetic acid was added dropwise to the solution at temperature of from −5° C. to −3° C. followed by stirring for one hour at 0°-3° C. The reaction mixture was evaporated to dryness under reduced pressure and ether was added to the residue

formed. The precipitates were recovered by filtration and washed well with ether and dried over phosphorus pentoxide under reduced pressure to provide 0.17 g. of 7β-{4-[(carboxy)(methylthio)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO)
δ(p.p.m.): 1.16 (3H, CH_3S—), 3.43 (3H, —OCH_3), 3.62 (2H, —CH_2— of C_2),

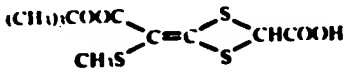


3.94 (3H,), 4.30 (—CH_2S— of C_3),
5.09 (1H, H of C_6),



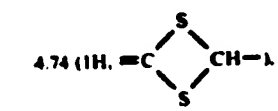
5.16 (1H,), 9.65 (1H, —CONH—).

REFERENCE EXAMPLE 4



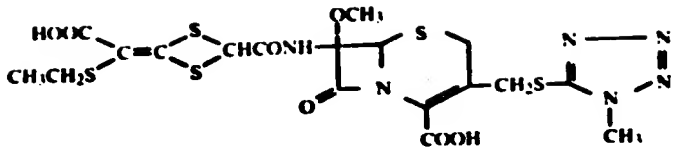
In 14 ml. of anhydrous tetrahydrofuran was suspended 0.96 g. of sodium hydride (50% in oil). After adding dropwise a mixture of 20 ml. of tert-butanol and 15 ml. of anhydrous tetrahydrofuran to the suspension, the mixture was stirred for 10 minutes at room temperature. Then, to the mixture was added a mixture of 1.62 g. of tert-butyl methylthioacetate and 5 ml. of anhydrous tetrahydrofuran at 3°-5° C. and after 30 minutes, 0.6 ml. of carbon disulfide was added to the mixture at the same temperature followed by stirring for 50 minutes. Then, 3.34 g. of sodium diiodoacetate was added to the mixture at temperature below 7° C. and they were caused to react for 50 minutes under ice-cooling. The solvent was distilled off under reduced pressure, the residue formed was dissolved in 50 ml. of ice-water, and the solution was washed twice each with ether. The aqueous layer formed was recovered, adjusted to pH 2 with 10% hydrochloric acid, dried over anhydrous magnesium sulfate, and then ether was distilled off under reduced pressure. The residue was subjected to a silica gel column chromatography and 1.3 g. of oily 4-[(tert-butoxycarbonyl)(methylthio)methylene]-1,3-dithietane-2-carboxylic acid using a mixture of chloroform, methanol, and formic acid of 95:5:2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$)
δ(p.p.m.): 1.52 (9H, $(CH_3)_3COOC$—), 2.22 (3H, CH_3S—),



4.74 (1H,), 9.12 (1H, —COOH).

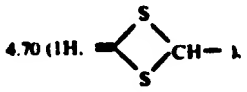
EXAMPLE 9



(a). By treating 0.15 g. of 4-[(tert-butoxycarbonyl)(ethylthio)methylene]-1,3-dithietane-2-carboxylic acid, 0.1 g. of N,N'-dicyclohexylcarbodiimide, and 0.26 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid benzhydryl ester as in Example 8-(a), 0.14 g. of 7β-{4-[(tert-butoxycarbonyl)(ethylthio)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid benzhydryl ester was obtained.

Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 1.22 (3H, CH_3CH_2S—), 1.52 (9H, tert-butyl), 2.68 (2H, CH_3CH_2S—), 3.58 (5H, —CH_2— of C_2 and —OCH_3), 3.82 (3H, —OCH_3), 4.38 (2H, —CH_2— of C_2),

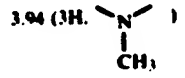


5.08 (1H, H of C_6),
6.92 (1H, —$CH(C_6H_5)_2$),
7.32 (10H, —$CH(C_6H_5)_2$),
7.79 (1H, —CONH—).

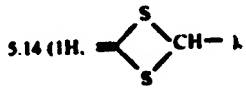
(b). By treating a mixture of 0.14 g. of the product obtained in step (a), 1.5 ml. of anisole, and 5 ml. of trifluoroacetic acid as in Example 8-(b), 0.07 g. of 7β-{4-[(carboxy)(ethylthio)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid was obtained.

Nuclear magnetic resonance spectra (D_6-DMSO)

δ(p.p.m.): 1.14 (3H, CH_3CH_2S—), 2.62 (2H, CH_3CH_2S—), 3.43 (3H, —OCH_3), 3.61 (2H, —CH_2— of C_2),

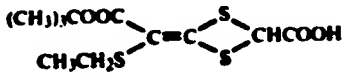


4.28 (2H, —CH_2S— of C_3),
5.08 (1H, H of C_6),



9.64 (1H, —CONH—).

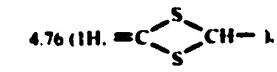
REFERENCE EXAMPLE 5



By treating 3.4 g. of tert-butyl ethylthioacetate as in Reference example 4, 4.05 g. of oily 4-[(tert-butoxycar-

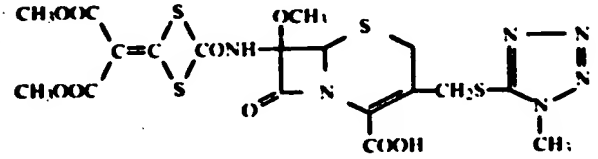
bonyl)(ethylthio)methylene]-1,3-dithietane-2-carboxylic acid was obtained.

Nuclear magnetic resonance spectra ($CDCl_3$)
δ(p.p.m.): 1.42 (3H, $C\underline{H}_3CH_2S$—), 1.52 (9H, $(CH_3)_3COOC$ –), 2.68 (2H, $CH_3C\underline{H}_2S$—),



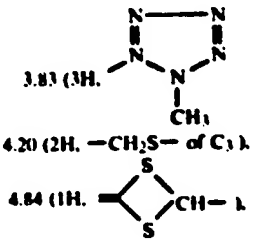
9.52 (1H, —COOH).

EXAMPLE 10



In 5 ml. of methylene chloride was dissolved 400 mg. of 4-[bis(methoxycarbonyl)methylene]-1,3-dithietane-2-carboxylic acid. After adding thereto 180 mg. of pyridine and further 290 mg. of phosphorus pentachloride under ice-cooling, the mixture was stirred for 30 minutes. The solution was added to a solution prepared by dissolving 500 mg. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester in 10 ml. of methylene chloride and cooling the solution to −20° C. to −30° C. The mixture was then stirred for one hour at the same temperature. The reaction mixture was washed successively with 10 ml. of water, 5 ml. of diluted hydrochloric acid, and 5 ml. of water, then dried over anhydrous magnesium sulfate, and distilled under reduced pressure. The residue was subjected to a silica gel column chromatography to provide 450 mg. of 7β-[4-{bis(methoxycarbonyl)methylene}-1,3-dithietan-2-yl]-carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of chloroform and ethyl acetate of 6:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$)
δ(p.p.m.): 3.58 (5H, CH_3O— of C_7 and —CH_2— of C_2), 3.80 (6H, —$COOCH_3$),



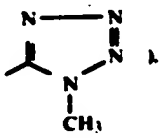
5.07 (1H, H of C_6),
6.93 (1H, —$C\underline{H}(C_6H_5)_2$),

7.36 (10H, H of phenyl of $-CH(C_6H_5)_2$).

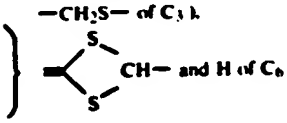
In a mixture of 4 ml. of trifluoroacetic acid and 1 ml. of anisole was dissolved 400 mg. of 7β-[4-{bis(methoxycarbonyl)methylene}-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester. The solution was stirred for one hour under ice-cooling. The reaction mixture was evaporated to dryness and the residue was mixed with ether. The product was recovered by filtration, washed well with ether, and dried overnight over phosphorus pentoxide under reduced pressure to provide 200 mg. of 7β-[4-{bis(methoxycarbonyl)methylene}-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO)

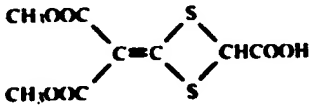
δ(p.p.m.): 3.44 (3H, CH_3O- of C_7), 3.70 (6H, $-COOCH_3$),

3.93 (3H, ),

4.29 (2H, $-CH_2S-$ of C_3),

5.08 (1H), 5.16 (1H) 

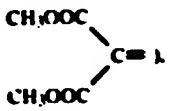
REFERENCE EXAMPLE 6

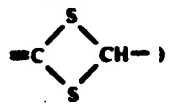


In 10 ml. of anhydrous tetrahydrofuran was suspended 2.1 g. of disodium 2,2-bis(methoxycarbonyl)ethylene-1,1-dithiolate.

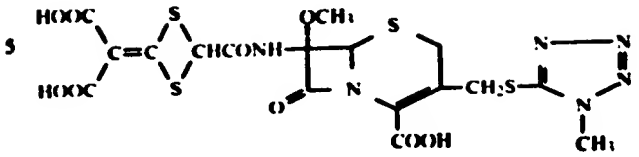
After adding 2.2 g. of sodium dibromoacetate to the suspension, the mixture was stirred for 2 hours at room temperature. The solvent was distilled off from the reaction mixture under reduced pressure and the residue was dissolved in 5 ml. of water. The solution was adjusted to pH 3.5–4.0 with diluted hydrochloric acid and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate and the solvent was distilled off under reduced pressure. The residue was mixed with ether and filtered to provide 1.5 g. of 4-[bis(methoxycarbonyl)methylene]-1,3-dithietane-2-carboxylic acid.

Nuclear magnetic resonance spectra (D_6DMSO)

δ (p.p.m.): 3.70 (6H, ),

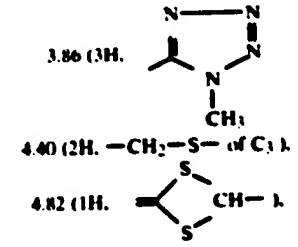
5.20 (1H, )

EXAMPLE 11



(a). In 5 ml. of methylene chloride was dissolved 500 mg. of [4-bis(tert-butoxycarbonyl)methylene-1,3-dithietan-2-yl]carboxylic acid. Then after adding 226 mg. of pyridine and further 360 mg. of phosphorus pentachloride to the solution under ice-cooling, the mixture was stirred for 30 minutes. The mixture was added to a solution prepared by dissolving 500 mg. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)-thiomethyl-Δ^3-cephem-4-caboxylic acid benzhydryl ester in 10 ml. methylene chloride and cooling to a temperature of from −20° C. to −30° C. and the mixture was stirred for one hour at the same temperature. The reaction mixture was washed successively with 10 ml. of water, 5 ml. of diluted hydrochloric acid, and 5 ml. of water, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure. The residue was subjected to a silica gel column chromatography using a mixture of chloroform and ethyl acetate of 6:1 by volume ratio as the eluant to provide 300 mg. of 7β-[4-{bis(tert-butoxycarbonyl)methylene}-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester.

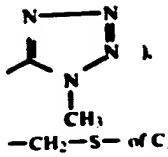
Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.50 (18H, t—C_4H_9), 3.60 (5H, CH_3O— of C_7 and —CH_2— of C_2),



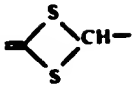
5.10 (1H, H of C_6), 6.94 (1H, —CH(C_6H_5)$_2$), 7.38 (10H, H of the phenyl of —CH(C_6H_5)$_2$).

(b). In a mixture of 4 ml. of trifluoroacetic acid and 0.5 ml. of anisole was dissolved 200 mg. of 7β-[4-{bis(-tert-butoxycarbonyl)methylene}-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester. The solution was stirred for one hour under ice-cooling. Then, the solvents were distilled off under reduced pressure and ether was added to the residue formed to form precipitates which were recovered by filtration. By washing the precipitates with ether, 100 mg. of 7β-{4-(dicarboxymethylene)-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid was obtained.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.44 (3H, CH_3O— of C_7), 3.64 (2H, —CH_2— of C_2),

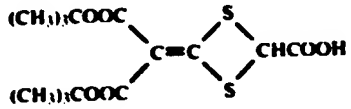
3.93 (3H. 

4.30 (2H. —CH_2—S— of C_3),
5.16 (1H)

H of C_6 and 

5.24 (1H)

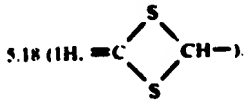
REFERENCE EXAMPLE 7



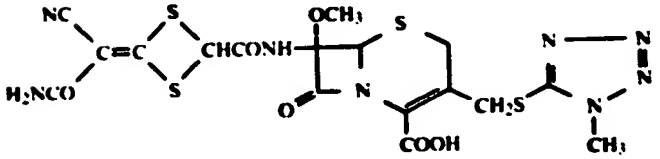
By following the same procedure as in Reference example 6 using disodium 2,2-bis(tert-butoxycarbonyl-)ethylene-1,1-dithiolate, 4-[bis(tert-butoxycarbonyl)methylene]-1,3-dithietane-2-carboxylic acid was obtained.

Nuclear magnetic resonance spectra (D_6-DMSO)

δ(p.p.m.): 1.46 (9H. $(CH_3)_3COOC$—),

5.18 (1H. 

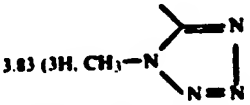
EXAMPLE 12



(a). In 12 ml. of tetrahydrofuran were dissolved 370 mg. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester, 150 mg. of N,N'-dicyclohexylcarbodiimide, and 150 mg. of 4-[(carbamoyl)(cyano)methylene]-1,3-dithietane-2-carboxylic acid followed by stirring for 2 hours at room temperature. The precipitates which had formed were filtered off and the solvent was distilled off from the filtrate under reduced pressure. The residue was subjected to a silica gel column chromatography using a mixture of chloroform and iso-propanol of 9:1 by volume ratio as the eluant to provide 190 mg. of 7β-[4-{(carbamoyl)(cyano)methylene}-1,3-dithietan-2-ylcarboxamido]-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester.

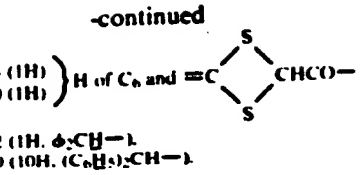
Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 3.55 (3H. 2H.—CH_3O and —CH_2— of C_2),



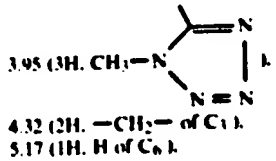
4.32 (2H. —CH_2— of C_3),

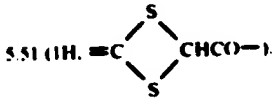
-continued



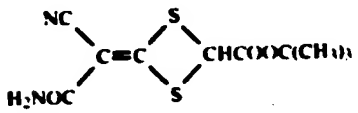
(b). In 10 ml. of methylene chloride was dissolved 160 mg. of the product obtained in above step (a). After adding thereto 0.5 ml. of anisole, the mixture was cooled to −20° C. Then, after adding dropwise 25 ml. of trifluoroacetic acid to the mixture at a temperature of from −20° C. to −10° C., the mixture was stirred for one hour at −10° C. to 0° C. The solvent was distilled off under reduced pressure and after adding 15 ml. of ether to the residue formed, the mixture was stirred for 20 minutes. Then, the mixture was filtered under reduced pressure and the precipitates thus obtained were washed well with ether and dried under reduced pressure to provide 80 mg. of 7β-[4-}(carbamoyl)(cyano)-methylene}-1,3-dithietan-2-yl carboxamido]-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.44 (3H, CH_3O—), 3.84 (2H, —CH_2— of C_2),





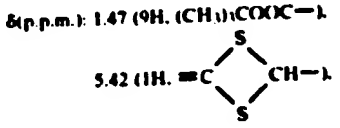
REFERENCE EXAMPLE 8



In 50 ml. of dimethyl sulfoxide was dissolved 4.8 g. of disodium 2-carbamoyl-2-cyano-ethylene-1,1-dithiolate. After adding 6.28 g. of tert-butyl dibromoacetate to the solution, the mixture was stirred for 48 hours at room temperature. The solvent was distilled off from the reaction mixture obtained under reduced pressure and the product was extracted with ethyl acetate. The extract was washed with water and then an aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure. The residue was subjected to a silica gel column chromatography and 0.8 g. of tert-butyl 4-[(carbamoyl)(cyano)methylene]-1,3-dithietane-2-car-

boxylate using a mixture of chloroform and ethyl acetate of 7:1 by volume ratio as the eluant.

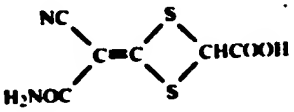
Nuclear magnetic resonance spectra (D_6-DMSO)



δ(p.p.m.): 1.47 (9H, $(CH_3)_3COOC-$),

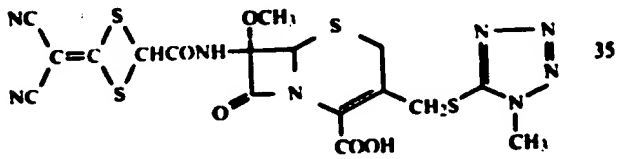
5.42 (1H, =C(S)CH(S)—),

REFERENCE EXAMPLE 9



To 0.4 g. of tert-butyl 4-[(carbamoyl)(cyano)methylene]-1,3-dithietan-2-carboxylate obtained in Reference example 7 were added 2 ml. of anisole and 8 ml. of trifluoroacetic acid. The mixture was then stirred for one hour at room temperature. The solvents were distilled off under reduced pressure and the residue was mixed with 10 ml. of ether followed by stirring for one hour. The precipitates thus formed were recovered by filtration, washed with ether, and dried under reduced pressure to provide 0.15 g. of 4-[(carbamoyl)(cyano)methylene]-1,3-dithietane-2-carboxylic acid.

EXAMPLE 13

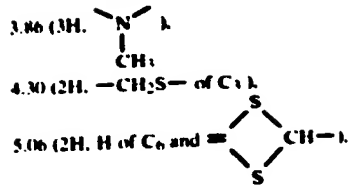


(a). In 12 ml. of methylene chloride was dissolved 0.43 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester. After cooling the solution to −40° C., 0.65 g. of pyridine was added thereto. Then, a solution prepared by dissolving 0.2 g. of 4-(dicyanomethylene)-1,3-dithietane-2-carboxylic acid in 8 ml. of methylene chloride, adding 0.21 g. of phosphorus pentachloride, and stirring the mixture for 25 minutes at room temperature was added dropwise to the above-prepared mixture at a temperature of from −40° C. to −25° C. and then the mixture was stirred for one hour at −30° C. to −20° C. After the reaction was over, 60 ml. of chloroform was added to the reaction mixture and the mixture was washed with 1% hydrochloric acid, water, and then a saturated aqueous sodium chloride solution. The organic layer formed was recovered and dried over anhydrous magnesium sulfate. The solvents were distilled off under reduced pressure and the residue was subjected to a silica gel column chromatography to provide 0.37 g. of 7β-[4-(dicyanomethylene)-1,3-dithietan-2-yl]-carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of chloroform and iso-propanol of 40:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 3.54 (5H, $-CH_2-$ of C_2 and $-OCH_3$),

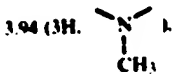
-continued



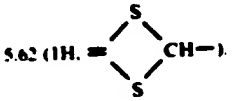
6.90 (1H, —CH(C$_6$H$_5$)$_2$)
7.30 (10H, —CH(C$_6$H$_5$)$_2$).

(b). In 10 ml. of methylene chloride was dissolved 0.37 g. of the product obtained in above step (a). After adding 0.5 ml. of anisole to the solution, the mixture was cooled to −20° C. Then, 2 ml. of trifluoroacetic acid was added dropwise to the mixture at −20° C. to −10° C. and the resultant mixture was stirred for 30 minutes at −10° C. to −5° C. The solvent was distilled off under reduced pressure and 20 ml. of ether was added to the residue followed by stirring for 30 minutes. The mixture was filtered under reduced pressure and the precipitates obtained were washed well with ether and dried under reduced pressure to provide 0.21 g. of 7β-[4-(dicyanomethylene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

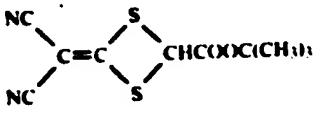
Nuclear magnetic resonance spectra (D$_6$-DMSO) δ(p.p.m.): 3.44 (3H, —OCH$_3$), 3.64 (2H, —CH$_2$— of C$_2$),



4.30 (2H, —CH$_2$S— of C$_3$), 5.18 (1H, H of C$_6$),



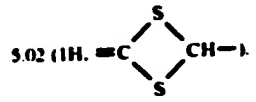
REFERENCE EXAMPLE 10



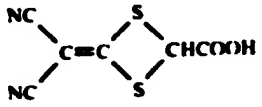
To 15 ml. of methylene chloride was added 0.28 g. of tert-butyl 4-[(carbamoyl)(cyano)methylene]-1,3-dithietane-2-carboxylate obtained in Reference example 7. After adding thereto 0.33 g. of pyridine and 0.43 g. of phosphorus pentachloride, the mixture was stirred for 30 minutes at room temperature. Then, 30 ml. of chloroform was added to the reaction mixture and the mixture was washed with 1 normal sulfuric acid, a 5% aqueous sodium carbonate solution, and then a saturated aqueous sodium chloride solution. The mixture was then dried over anhydrous magnesium sulfate and the solvent was distilled off under reduced pressure. The residue formed was subjected to a silica gel column chromatography to provide 0.23 g. of tert-butyl 4-dicyanomethylene-1,3-dithietan-2-carboxylate using chloroform as the eluant.

Nuclear magnetic resonance spectra (CDCl$_3$)

δ(p.p.m.): 1.54 (9H, —COOC(CH$_3$)$_3$)

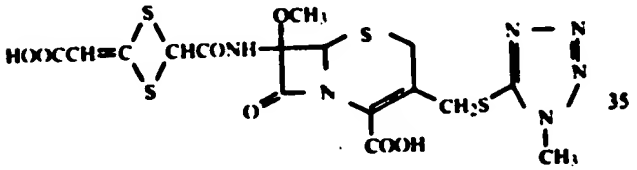


REFERENCE EXAMPLE 11



To 0.23 g. of tert-butyl 4-dicyanomethylene-1,3-dithietane-2-carboxylate obtained in Reference example 9 were added 2 ml. of anisole and 6 ml. of trifluoroacetic acid. And the mixture was stirred for 3 hours at room temperature. The solvents were distilled off under reduced pressure and 10 ml. of hexane was mixed with the residue followed by stirring for 10 minutes. The solvent was removed by decantation. Then the same procedure was applied twice to the residue thus formed. The residue was then dried under reduced pressure to provide 0.18 g. of 4-dicyanomethylene-1,3-dithietane-2-carboxylic acid.

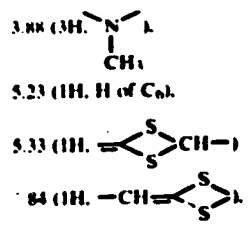
EXAMPLE 14



(a). In 20 ml. of methylene chloride was dissolved 0.714 g. of 4-(tert-butoxycarbonylmethylene)-1,3-dithietane-2-carboxylic acid. Then 0.454 g. of pyridine was added to the solution followed by cooling to a temperature below 5° C. Thereafter, 0.630 g. of phosphorus pentachloride was added to the mixture to cause the reaction for one hour at a temperature below 10° C. The reaction mixture obtained was cooled to about −50° C. and a solution prepared by dissolving 1.5 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester in 15 ml. of methylene chloride was added dropwise to the reaction mixture. Then, 3 ml. of pyridine was added and the reaction was performed for 1 hour at −30° C. to −35° C. After the reaction was over, 20 ml. of 6 normal hydrochloric acid was added to the reaction mixture at a temperature below 0° C. The methylene chloride layer formed was recovered and the aqueous layer was further extracted with 20 ml. of methylene chloride. The extract was combined with the methylene chloride layer and the mixture was washed twice each time with 20 ml. of a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then the solvent was distilled off to provide 1.89 g. of a brown caramel residue. The residue was subjected to a silica gel column chromatography to provide 0.308 g. of 7β-[(4-tert-butoxycarbonylmethylene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl

ester using a mixture of ethyl acetate and n-hexane of 2:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 1.40 (9H, tert-butyl), 3.44 (3H, —OCH_3),

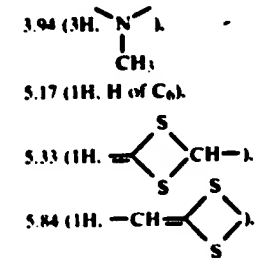


6.88 (1H, —$CH(C_6H_5)_2$)

9.66 (1H, —CONH—).

(b). In 1.7 ml. of anisole was dissolved 0.3 g. of the product obtained in aforesaid step (a). After cooling the solution to a temperature below −5° C., 5.1 ml. of trifluoroacetic acid was added dropwise to the solution at a temperature below 0° C. Thereafter, the reaction was performed for 30 minutes at 0°-5° C. and then for 30 minutes at 5°-10° C. After the reaction was over, anisole and trifluoroacetic acid were distilled off under reduced pressure and the residue was powdered with the addition of ether. The powder was washed well with ether, and dried to provide 0.1584 g. of faint-yellow powdery (7β-[4-(carboxymethylene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.43 (3H, —OCH_3),



9.63 (1H, —CONH—)

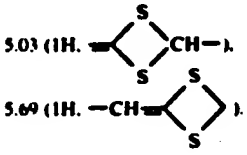
REFERENCE EXAMPLE 12

A mixture of 80 ml. of dimethoxyethane and 20 ml. of tetrahydrofuran deoxygenated by distillation was cooled below −70° C. in a nitrogen stream and after adding thereto 2 ml. of N-isopropylcyclohexylamine and 6.86 ml. of a 15% n-butyl lithium n-hexane solution, 1.16 g. of tert-butyl acetate was added dropwise to the mixture. Then, the reaction was performed for 30 minutes at a temperature below −70° C. and then 0.664 ml. of carbon disulfide was added to the reaction mixture over a period of about 30 minutes at a temperature below −72° C. The reaction mixture colored light yellow. After further causing the reaction for 20 minutes at a temperature below −70° C., 6.8 ml. of 15% n-butyl lithium n-hexane solution was added dropwise to the reaction mixture over a period of 15 minutes at a temperature below −72° C. Thereafter, the reaction was further performed for 20 minutes at a temperature below −70° C. and then a solution containing crystals of sodium diiodoacetate prepared from 0.48 g. of 50%

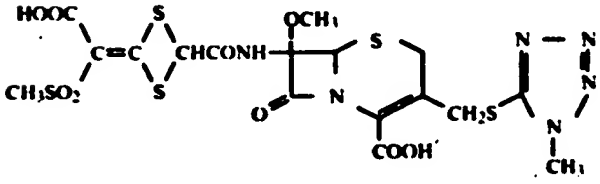
sodium hydride and 3.12 g. of diiodoacetic acid in 15 ml. of dimethoxyethane was added to the reaction mixture. The temperature of the reaction mixture was allowed to raise to room temperature and the reaction mixture was further reacted overnight. The solvent was distilled off and the black-brown oily material obtained was extracted with the additions of 50 ml. of cold ether and 20 ml. of 1 normal hydrochloric acid.

The aqueous layer was further extracted with the addition of 30 ml. of cold ether and the extracts were combined. The mixture was washed twice each time with 30 ml. of a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then ether was distilled off to provide 3 g. of a brown oily product. The product was subjected to a silica gel column chromatography to provide 0.564 g. of 4-(tert-butoxycarbonylmethylene)-1,3-dithietane-2-carboxylic acid using a mixture of chloroform, methanol, and formic acid of 95:5:2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 1.45 (9H, tert-butyl),

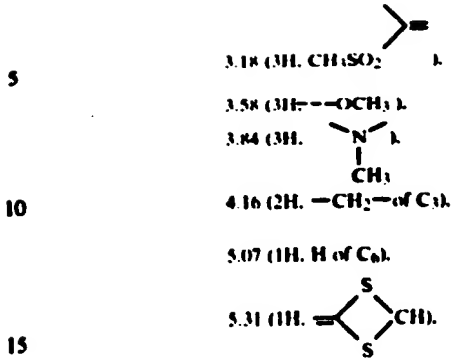


EXAMPLE 15



(a). In 8 ml. of methylene chloride was dissolved 0.32 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester. After cooling the solution to −30° C., 0.48 g. of pyridine was added to the solution. Then, a solution prepared by dissolving 0.37 g. of 4-[(tert-butoxycarbonyl)(methylsulfonyl)methylene]-1,3-dithietane-2-carboxylic acid in 8 ml. of methylene chloride and adding thereto 0.25 g. of phosphorus petachloride and 0.18 g. of pyridine was added dropwise to the solution at a temperature of −40° C. to −30° C. After stirring the mixture for one hour at −30° C. to −20° C., 50 ml. of chloroform was added to the mixture and the resultant mixture was washed with 1% hydrochloric acid, water, and then a saturated aqueous sodium chloride solution, and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure and the residue was subjected to a silica gel column chromatography to provide 0.25 g. of 7β-{4-[(tert-butoxycarbonyl)-(methylsulfonyl)methylene]-1,3-dithietan-2-yl}-carboxamido-7α-methoxy-3- (1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of chloroform and isopropanol of 40:1 by volume ratio as the eluant.

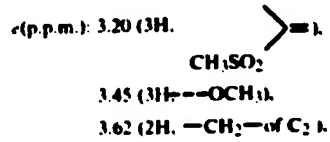
Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.54 (9H, tert-butyl)



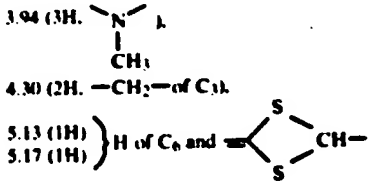
6.91 (1H, —CH(C$_6$H$_5$)$_2$),
7.30 (10H, —CH(C$_6$H$_5$)$_2$).

(b). To 2.5 ml. of anisole was added 0.2 g. of the product obtained in step (a). The solution was cooled to −20° C., and then 10 ml. of trifluoroacetic acid was added dropwise to the mixture at −20° C. to −10° C. Then, after stirring the mixture for 20 minutes at the same temperature, the mixture was further stirred for 40 minutes at 10° C. The solvent was distilled off under reduced pressure, the residue formed was mixed with 30 ml. of ether, and the mixture was stirred for 20 minutes. The reaction mixture was filtered under reduced pressure and the precipitates thus obtained were washed well with ether and dried under reduced pressure to provide 0.08 g. of 7β-{4-[(carboxy)(methylsulfonyl)-methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-ceph-em-4-carboxylic acid.

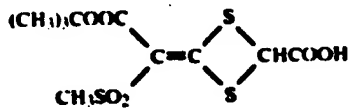
Nuclear magnetic resonance spectra (D$_6$-DMSO)



3.45 (3H, —OCH$_3$),
3.62 (2H, —CH$_2$— of C$_2$),



REFERENCE EXAMPLE 13



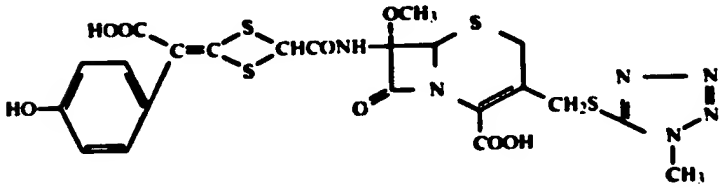
In 65 ml. of tert-butanol was dissolved 2.05 g. of tert-butyl methylsulfonylacetate. After adding thereto 1.32 g. of potassium tert-butylate, the mixture was stirred for 5 minutes. After adding dropwise 0.91 g. of carbon disulfide to the mixture and stirring them for 5

minutes, 1.32 g. of potassium tert-butylate was added to the mixture followed by stirring for one hour. Then, 3.8 g. of diiodoacetic acid and 1.32 g. of potassium tert-butylate were added to the mixture and the resultant mixture was stirred overnight. The solvent was distilled off from the reaction mixture obtained under reduced pressure. The residue formed was mixed with water, adjusted to pH 2 with 10% hydrochloric acid, and extracted with ethyl acetate. The extract was washed with water and then a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure. The residue was subjected to a silica gel column chromatography to provide 1.7 g. of 4-[(tert-butoxycarbonyl)(methylsulfonyl)methylene]-1,3-dithietane-2-carboxylic acid using a mixture of chloroform and methanol of 50:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 1.52 (9H, $(CH_3)_3COOC-$) 3.20 (3H, CH_3SO_2-).

EXAMPLE 16



(a). In 1.5 ml. of methylene chloride was dissolved 0.6 g. of 4-(4-tert-butoxy-α-tert-butoxycarbonylbenzylidene)-1,3-dithietane-2-carboxylic acid. After adding thereto 0.2 ml. of pyridine and further 0.285 g. of phosphorus pentachloride under ice-cooling, the mixture was stirred for 7 minutes. The solution was added to a solution prepared by dissolving 0.5 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester in 15 ml. of methylene chloride, cooling to −30° C. to −40° C., and adding thereto 0.45 ml. of pyridine, and the mixture was stirred for 20 minutes at the same temperature.

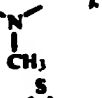
The reaction mixture was mixed with 60 ml. of chloroform, washed with about 30 ml. of water, about 30 ml. of 1-2% hydrochloric acid, and then 30 ml. of water, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure. The residue was subjected to a silica gel column chromatography to provide 0.4 g. of 7β-[4-(4-tert-butoxy-α-tert-butoxycarbonylbenzylidene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of benzene and ethyl acetate of 11:2 by volume ratio as the eluant.

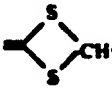
Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.):

1.35 (9H), 1.47 (9H) } tert-butyl

3.60 (5H, $-OCH_3$ and $-CH_2-$ of C_2),

3.83 (3H, ),

4.83 (1H, ),

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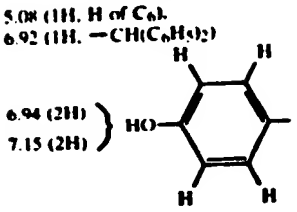
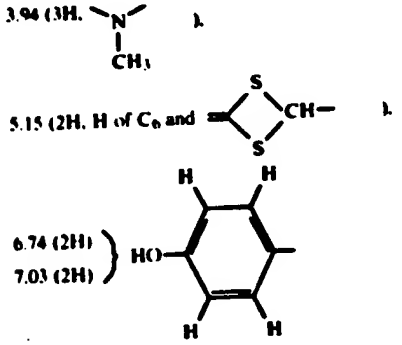
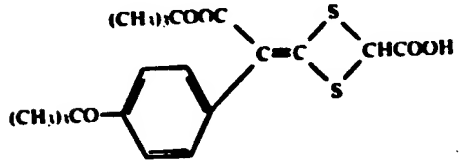


(b). In a mixture of 10 ml. of trifluoroacetic acid and 2 ml. of anisole was dissolved 0.4 g. of the product obtained in step (a) under ice-cooling. The mixture was stirred for about 30 minutes at 10° C. The solvent was distilled off under reduced pressure and 40 ml. of ether was added to the residue to form precipitates, which were recovered by filtration and washed with ether to provide about 0.2 g. of 7β-[4-(α-carboxy-4-hydroxybenzylidene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.42 (3H, —OCH_3),



REFERENCE EXAMPLE 14



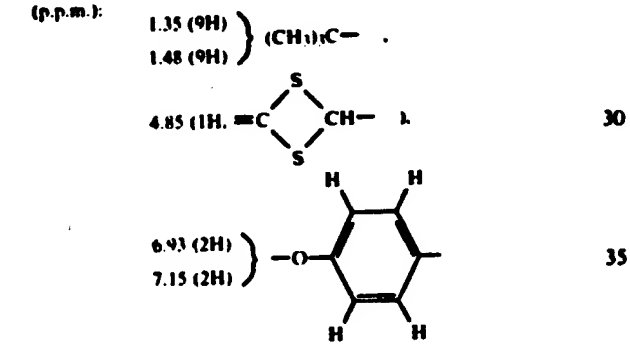
To 8.6 ml. of a 15% potassium tert-butylate tert-butanol solution were added 2.5 g. of tert-butyl 4-tert-butoxyphenylacetate and 25 ml. of anhydrous tetrahydrofuran with stirring at room temperature. After stirring the mixture for 2-3 minutes, 0.6 ml. of carbon disulfide was added dropwise to the mixture followed by stirring for 10 minutes. Then, 8.6 ml. of a 15% potassium tert-butylate tert-butanol solution was added to the

mixture followed by stirring for 5 minutes, further 8.6 ml. of a 15% potassium tert-butylate tert-butanol solution was added to the mixture and then 1.22 g. of dichloroacetic acid was added dropwise to the mixture at about 30° C. followed by stirring for 40 minutes at room temperature.

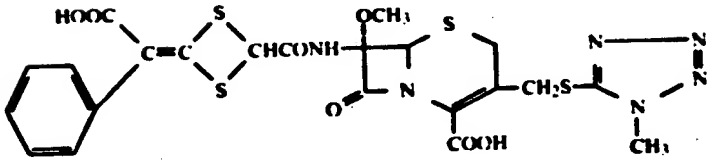
Then, after adding dichloroacetic acid to the reaction mixture until the mixture became weak alkaline, the solvent was distilled off under reduced pressure and the residue was mixed with ice-water followed by washing with ether. Then, 0.5 ml. of 3 normal hydrochloric acid was added to the mixture and the product was extracted with ether. To the extract was further added 0.5 ml. of 3 normal hydrochloric acid. The product was extracted with ether, and the procedure was further repeated. Each extract fraction obtained was detected by a silica gel thin layer chromatography, the fractions containing the product were collected and dried over anhydrous magnesium sulfate. Then, the solvent was distilled off under reduced pressure to provide 1.3 g. of 4-(4-tert-butoxy-α-tert-butoxycarbonylbenzilidene)-1,3-dithietane-2-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO)

(p.p.m.):



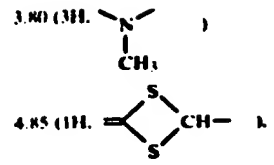
EXAMPLE 17



(a). In 15 ml. of methylene chloride was dissolved 0.5 g. of 4-(α-tert-butoxycarbonylbenzylidene)-1,3-dithietane-2-carboxylic acid. After adding thereto 0.2 ml. of pyridine and further 0.285 g. of phosphorus petachloride under ice-cooling, the mixture was stirred for about 20 minutes. Then, by treating the reaction mixture as in Example 16-(a) using, however, a mixture of benzene and ethyl acetate of 9:2 by volume ratio as the eluant for silica gel column chromatography, about 0.4 g. of 7β-[4-(α-tert-butoxycarbonylbenzylidene)-1,3-dithietane-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester was obtained.

Nuclear magnetic resonance spectra ($CDCl_3$)

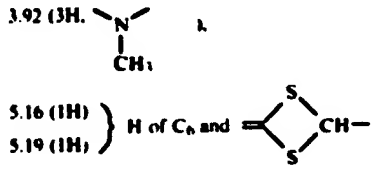
δ(p.p.m.): 1.46 (9H, tert-butyl), 3.58 (5H, OCH_3 and $—CH_2—$ of C_2),



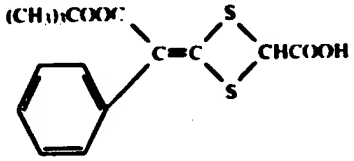
5.08 (1H, H of C_6),
6.93 (1H, —CH$(C_6H_5)_2$),
about 7.34 (15H, C_6H_5— and $(C_6H_5)_2$CH—).

(b). By treating 0.4 g. of the product obtained in above step (a) as in Example 16-(b), about 0.2 g. of 7β-[4-(α-carboxybenzylidene)-1,3-dithietane-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid was obtained.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.41 (3H, —OCH_3),



REFERENCE EXAMPLE 15

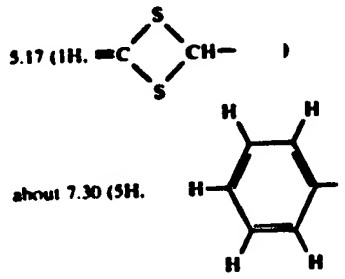


To 15.6 g. of a 15% potassium tert-butylate tert-butanol solution were added 4 g. of tert-butyl phenylacetate and then 1.6 g. of carbon disulfide with stirring at room temperature. After stirring the mixture for 15 minutes, 20 ml. of anhydrous tetrahydrofuran and then 31.2 g. of a 15% potassium tert-butylate tert-butanol solution were added to the mixture and then 2.7 g. of dichloroacetic acid was added dropwise to the mixture at 30°–40° C. followed by stirring for 30 minutes at the same temperature to finish the reaction.

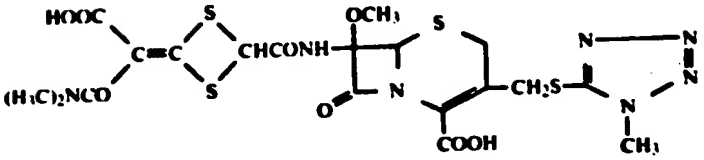
Then, after adding dichloroacetic acid to the reaction mixture until the mixture became weakly alkaline, the solvent was distilled off under reduced pressure and the residue was mixed with ice-water followed by washing with ether. Then, 0.5 ml. of 3 normal hydrochloric acid was added to the mixture and the product was extracted with ether. To the extract was further added 0.5 ml. of 3 normal hydrochloric acid. The product was extracted with ether, and the procedure was further repeated. Each extract fraction obtained was detected by a silica

gel thin layer chromatography, the fractions containing the objective material were collected and dried over anhydrous magnesium sulfate. Then, the solvent was distilled off under reduced pressure to provide about 1 g. of 4-(α-tert-butoxycarbonylbenzylidene)-1,3-dithietane-2-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 1.40 (9H, $(CH_3)_3COOC$—),



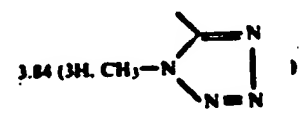
EXAMPLE 18



(a). In 15 ml. of methylene chloride was dissolved 500 mg. of 4-(tert-butoxycarbonyl-N,N-dimethylcarbamoylmethylene)-1,3-dithietane-2-carboxylic acid. After adding thereto 0.19 ml. of pyridine and further 163 mg. of phosphorus pentachloride under ice-cooling, the mixture was stirred for about 5 minutes. The solution was added to a solution prepared by dissolving 500 mg. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid in 15 ml. of methylene chloride and then adding 0.3 ml. of pyridine to the solution while cooling the solution to −30° C., and the mixture was stirred for about 30 minutes at the same temperature.

To the reaction mixture was added about 60 ml. of chloroform. The mixture was washed with about 30 ml. of water, about 30 ml. of 1-2% hydrochloric acid, and then three times each time with about 30 ml. of water, and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure and the residue formed was subjected to a silica gel column chromatography to provide 200 mg. of 7β-[(4-tert-butoxycarbonyl-N,N-dimethylcarbamoylmethylene)-1,3-dithietane-2-yl-carboxamido]-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of benzene and ethyl acetate of 3:2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.48 (9H, tert-C_4H_9—), 2.96 (6 Hm $(CH_3)_2NCO$—) 3.60 (5H, CH_3O and —CH_2— of C_2),



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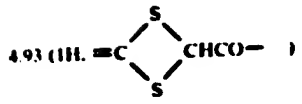
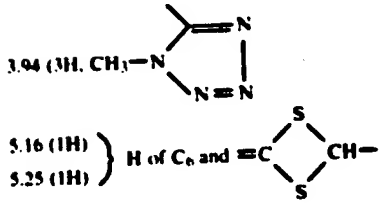


5.06 (1H, H of C_6),

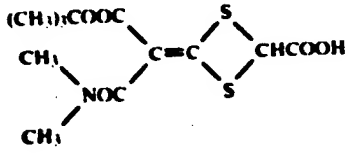
6.90 (1H, —C<u>H</u>$(C_6H_5)_2$).

(b). In a mixture of 10 ml. of trifluoroacetic acid and 2 ml. of anisole was dissolved the product obtained in above step (a) under ice-cooling. And the mixture was stirred for about 30 minutes at 15° C. The solvent was distilled off under reduced pressure, 30 ml. of ether was added to the residue, and the precipitates formed were recovered by filtration and washed with ether to provide 100 mg. of 7β-[(4-carboxy-N,N-dimethylcarbamoylmethylene)-1,3-dithietane-2-yl-carboxamido]-7α-methoxy-3-(1-methyltetrazole-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 2.87 (6H, $(CH_3)_2NCO$—), 3.43 (3H, CH_3O—),

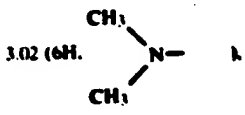


REFERENCE EXAMPLE 16



By treating tert-butyl dimethylcarbamoylacetate as in Reference example 15, 4-[(tert-butoxycarbonyl)(dimethylcarbamoyl)methylene]-1,3-dithietane-2-carboxylic acid was obtained.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.50 (9H, $(CH_3)_3COOC$—),



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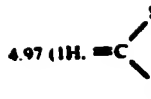
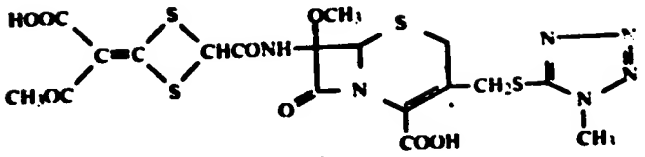
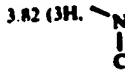
4.97 (1H. )

EXAMPLE 19

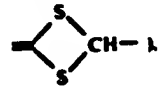


(a). In 15 ml. of methylene chloride was dissolved 0.87 g. of 4-[(acetyl)(tert-butoxycarbonyl)methylene]-1,3-dithietane-2-carboxylic acid. After adding thereto 0.474 g of pyridine and further 0.624 g. of phosphorus pentachloride under ice-cooling, the mixture was stirred for 30 minutes. The solution was added to a solution prepared by dissolving 0.6 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)-thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester in 20 ml. of methylene chloride, cooling the solution to −30° C., and adding thereto 1 ml. of pyridine, and the mixture was stirred for one hour at the same temperature. To the reaction mixture were added 10 ml. of water, 1 ml. of 1 normal hydrochloric acid, and 30 ml. of chloroform. The chloroform layer formed was recovered, washed thoroughly with 1% hydrochloric acid to eliminate pyridine completely, then with water, dried over anhydrous sodium sulfate, and the solvent was distilled off under reduced pressure. The residue formed was subjected to a silica gel column chromatography to provide 0.55 g. of 7β-{4-[(acetyl)(tert-butoxycarbonyl)-methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of benzene and ethyl acetate of 10:2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.53 (9H, tert-butyl), 2.45 (3H, CH_3OC—), 3.56 (5H, —OCH_3 and —CH_2— of C_2),

3.82 (3H. )

4.37 (2H. —CH_2S— of C_3),

4.94 (1H. )

5.09 (1H, H of C_6),

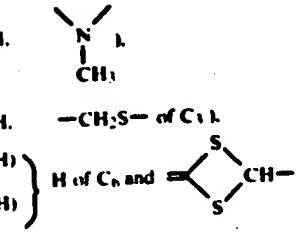
6.94 (1H, —C<u>H</u>$(C_6H_5)_2$),

7.20–7.50 (10H, —CH(C_6<u>H</u>$_5)_2$).

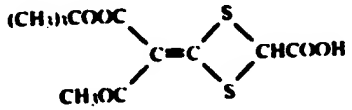
(b). In a mixture of 12 ml. of trifluoroacetic acid and 3 ml. of anisole was dissolved 0.55 g. of the product obtained in aforesaid step (a) at −5° C. The mixture was stirred for 20 minutes at 15° C. The solvent was distilled off under reduced pressure, 20 ml. of ether was added to the residue, and the precipitates formed were recovered by filtration and washed with ether to provide 0.33 g. of 7β-{4-[(acetyl)(carboxy)methylene]-1,3-dithietan-2-

yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 2.38 (3H, CH_3OC—), 3.43 (3H, —OCH_3),



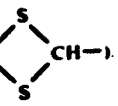
REFERENCE EXAMPLE 17



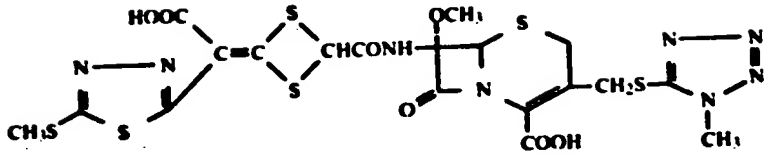
To 150 ml. of tert-butanol was added 4.8 g. of sodium hydride (50% in oil). Then 15.8 g. of tert-butyl acetoacetate was added gradually to the mixture. Then, after adding thereto 7.6 g. of carbon disulfide under ice-cooling, the mixture was stirred for 18 hours at room temperature. Thereafter, 4.8 g. of sodium hydride (50% in oil) was added gradually under ice-cooling and after stirring the mixture for 2 hours at room temperature, 16.7 g of potassium dichloroacetate was added to the mixture followed by stirring for further 2 hours. The reaction mixture obtained was concentrated under reduced pressure. The residue was mixed with 300 ml. of ethyl acetate and 200 ml. of ice-water, and the mixture was adjusted to pH 3–4 with 1 normal hydrochloric acid. The organic layer formed was washed with an aqueous sodium chloride solution, and extracted with a saturated aqueous sodium hydrogencarbonate solution.

The sodium hydrogencarbonate extract was washed with 50 ml. of ethyl acetate, adjusted to pH 3–4 with 1 normal hydrochloric acid, and extracted with 200 ml. of ethyl acetate. The ethyl acetate extract was washed with an aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and then concentrated. The residue was washed with 50 ml. of a mixture of petroleum ether and ether of 10:1 by volume ratio and dissolved in 5 ml. of ether. Then, 50 ml. of petroleum ether was added gradually to the solution and the crystals thus precipitated were recovered by filtration to provide 10 g. of 4-[(acetyl)(tert-butoxycarbonyl)methylene]-1,3-dithietane-2-carboxylic acid.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.53 (9H, $(CH_3)_3COOC$—), 2.49 (3H, CH_3OC—),

4.94 (1H, =C  CH—)

EXAMPLE 20

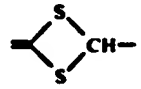


(a). In 20 ml. of methylene chloride was dissolved 1.1 g. of 4-[(tert-butoxycarbonyl)(5-methylthio-1,3,4-thiadiazol-2-yl)methylene]-1,3-dithietane-2-carboxylic acid. After adding thereto 0.462 g. of pyridine and further 0.606 g. of phosphorus pentachloride under ice-cooling, the mixture was stirred for 30 minutes. The solution was added to a solution prepared by dissolving 0.9 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester in 30 ml. of methylene chloride, cooling the solution to −30° C., and adding 0.75 ml. of pyridine to the solution, and the mixture was stirred for one hour at room temperature. The reaction mixture was mixed with 10 ml. of water and 40 ml. of chloroform. Then, the chloroform layer formed was washed thoroughly with 1% hydrochloric acid to eliminate pyridine completely, then with water, dried over anhydrous sodium sulfate, and then the solvent was distilled off under reduced pressure. The residue was subjected to a silica gel column chromatography to provide 0.2 g. of 7β-{4-[(tert-butoxycarbonyl)(5-methylthio-1,3,4-thiadiazol-2-yl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of benzene and ethyl acetate of 10:2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.58 (9H, tert-butyl), 2.72 (3H, CH_3S—), 3.58 (3H, —OCH_3),

3.80 (3H, )

4.36 (2H —CH_2S— of C_3),

5.03 (1H), 5.09 (1H) } H of C_6 and 

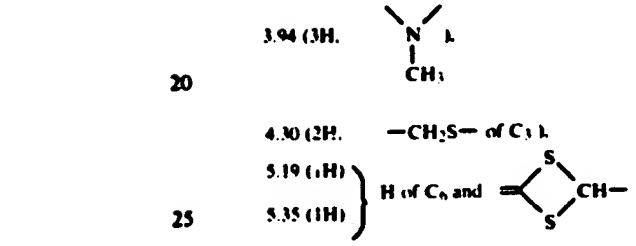
6.90 (1H, —C<u>H</u>$(C_6H_5)_2$),

7.10–7.50 (10H, —CH(C_6<u>H</u>$_5)_2$).

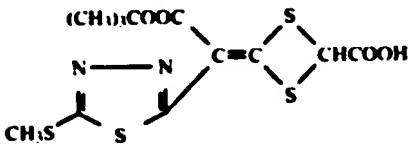
(b). In a mixture of 8 ml. of trifluoroacetic acid and 2 ml. of anisole was dissolved 0.2 g. of the product obtained in above step (a) at −5° C. followed by stirring for one hour at 17°–18° C. The solvent was distilled off under reduced pressure, 20 ml. of ether was added to the residue, and the precipitates formed were recovered by filtration and washed with ether to provide 0.05 g. of 7β-{4-[(carboxy)(5-methylthio-1,3,4-thiadiazol-2-yl)methylene]-1,3-dithietan-2-yl}-carboxamido-7α-

methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 2.75 (3H, CH_3S—), 3.45 (3H, —OCH_3),

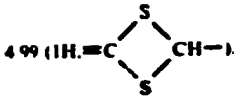


REFERENCE EXAMPLE 18

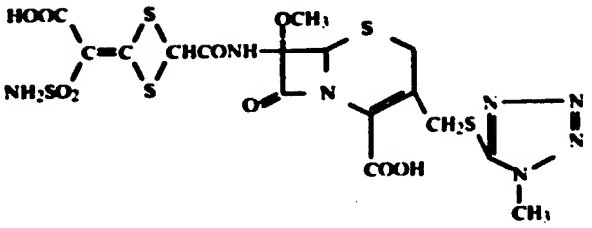


In 100 ml. of tert-butanol was dissolved 1.58 g. of metallic potassium. After adding thereto 10 g. of tertbutyl 5-methylthio-1,3,4-thiadiazole-2-acetate, the mixture was stirred for 20 minutes. Thereafter, 3.25 g. of carbon disulfide was added dropwise to the mixture over a period of 10 minutes. After stirring the mixture for one hour, 4.55 g. of potassium tert-butyrate was added gradually to the mixture followed by stirring for 20 minutes and then 6.83 g. of potassium dichloroacetate was added to the mixture followed by stirring for 18 hours. The reaction mixture was concentrated under reduced pressure, and the residue was mixed with 300 ml. of ethyl acetate and 200 ml. of ice-water. The mixture was adjusted to pH 3–4 with 1 normal hydrochloric acid, and the organic layer formed was washed with an aqueous sodium chloride solution, and then extracted with 1000 ml. of a saturated aqueous sodium hydrogencarbonate solution. The sodium hydrogencarbonate extract was washed with 100 ml. of ethyl acetate, adjusted to pH 3–4 with 5 normal hydrochloric acid, and then extracted with 200 ml. of ethyl acetate. The ethyl acetate extract was washed with an aqueous sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated. The residue was subjected to a silica gel column chromatography to provide 1 g. of 4-[(tert-butoxycarbonyl)(5-methylthio-1,3,4-thiadiazol-2-yl)methylene]-1,3-dithietane-2-carboxylic acid using chloroform and then a mixture of chloroform and methanol of 50:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.59 (9H, $(CH_3)_3COOC$—), 2.79 (3H, CH_3S—),



EXAMPLE 21

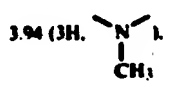


(a). In 12 ml. of anhydrous tetrahydrofuran were dissolved 0.2 g. of 4-[(benzhydryloxycarbonyl)(sulfamoyl)methylene]-1,3-dithietane-2-carboxylic acid, 0.25 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester, and 0.1 g. of N,N'-dicyclohexylcarbodiimide.

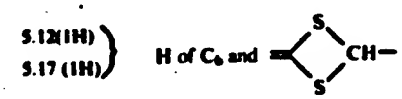
Then the solution was stirred overnight at room temperature. Insoluble materials were filtered off and the solvent was distilled off from the filtrate under reduced pressure. The residue was subjected to a silica gel column chromatography to provide 0.095 g. of 7β-{4-[(benzhydryloxycarbonyl)(sulfamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of chloroform and isopropanol of 9:1 by volume ratio as the eluant.

(b). In 10 ml. of methylene chloride was dissolved 0.095 g. of the product obtained in step (a). After adding thereto 0.5 ml. of anisole, the mixture was cooled to −20° C. Thereafter, 2 ml. of trifluoroacetic acid was added dropwise to the mixture at −20° C. to −15° C. and after stirring the mixture for 30 minutes at the same temperature, the resultant mixture was further stirred for one hour at 0°-3° C. The solvent was distilled off from the reaction mixture under reduced pressure and 15 ml. of ether was added to the residue followed by stirring for 30 minutes. Then, the reaction mixture was filtered under reduced pressure and the precipitates formed were washed well and dried under reduced pressure to provide 0.034 g. of 7β-{4-[(carboxy)(sulfamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.42 (3H, —OCH_3),

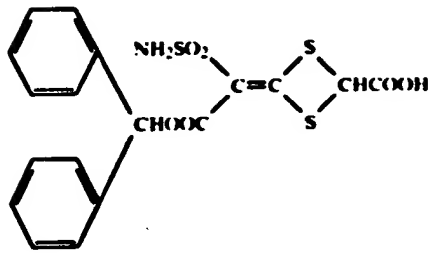


4.30 (2H, —CH_2— of C_3),



9.66 (1H, —NHCO—).

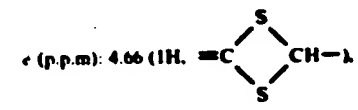
REFERENCE EXAMPLE 19



To 1.12 g. of benzhydryl sulfamoylacetate were added 30 ml. of anhydrous tetrahydrofuran and 20 ml. of tert-butanol.

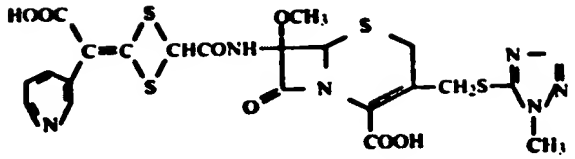
After cooling the mixture to −20° C., 0.177 g. of sodium hydride (50% in oil) was added to the mixture followed by stirring for 15 minutes. To the mixture was added 0.3 g. of carbon disulfide. The mixture was stirred for 30 minutes at −10° C. to −5° C. Then, to the mixture were added 0.354 g. of sodium hydride (50% in oil) and 1.05 g. of diiodoacetic acid. After stirring the mixture for 20 minutes at −10° C. to 0° C., the mixture was stirred overnight at room temperature. The solvent was distilled off from the reaction mixture under reduced pressure and after adjusting the residue to pH 2 by adding thereto ice-water and 5% hydrochloric acid, the reaction mixture was extracted with ethyl acetate. The extract was washed twice each time with a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then the solvent was distilled under reduced pressure. The residue was subjected to a silica gel column chromatography to provide 0.2 g. of 4-[(benzhydryloxycarbonyl)(sulfamoyl)methylene]-1,3-dithietane-2-carboxylic acid using a mixture of chloroform and methanol of 10:1 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCl_3$)



6.96 (1H, $(C_6H_5)_2C\underline{H}$—)
7.33 (10H, $(C_6\underline{H}_5)_2CH$—)

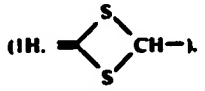
EXAMPLE 22



(a). In 40 ml. of methylene chloride was dissolved 800 mg. of 4-[(tert-butoxycarbonyl)(3-pyridyl)methylene]-1,3-dithietane-2-carboxylic acid. After adding thereto 0.3 ml. of pyridine and further 440 mg. of phosphorus pentachloride under ice-cooling, the mixture was stirred for about 15 minutes. The solution was added to a solution prepared by dissolving 0.8 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid in 25 ml. of methylene chloride and adding thereto 0.7 ml. of pyridine at −30° C., and the resultant mixture was stirred for 20 minutes at the same temperature. Then, 200 ml. of chloroform was added to the reaction mixture and the mixture was washed twice each time with 150 ml. of an aqueous 1.3% acetic acid solution and then twice each time with 100 ml. of water, and then dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure and the residue formed was subjected to a silica gel column chromatography to provide about 400 mg. of 7β-{4-[(tert-butoxycarbonyl)(3-pyridyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid using a mixture of benzene and ethyl acetate of 2:1 by volume ratio as the eluant.

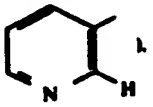
Nuclear magnetic resonance spectra (CD Cl_3) δ(p.p.m.): 1.47 (9H, t-C_4H_9—), 3.59 (5H, CH_3O— and —CH_2— of C_2),

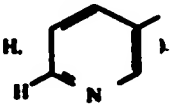
3.83 (3H, $N(CH_3)_2$),

4.91 (1H, ),

5.08 (1H, H of C_6),

6.93 (1H, —$CH(C_6H_5)_2$),

8.50 (1H ),

8.53 (1H, ),

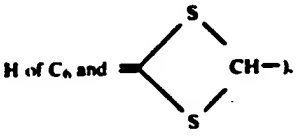
(b). In a mixture of 15 ml. of trifluoroacetic acid and 3 ml. of anisole was dissolved 400 mg. of the compound obtained in above step (a) under ice cooling followed by stirring for 40 minutes at 10°-15° C. The solvent was distilled off under reduced pressure and about 50 ml. of ether was added to the residue to form precipitates, which were recovered by filtration, and washed with

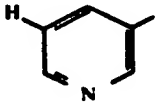
ether. The precipitates were subjected to a silica gel column chromatography to provide about 100 mg. of 7β-{[4-(carboxy)(3-pyridyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid using a mixture of chloroform, methanol, and formic acid of 50:7:1 by volume ratio as the eluant.

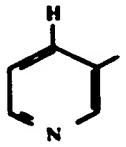
Nuclear magnetic resonance spectra (D_6-DMSO)

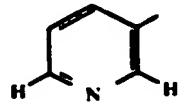
δ(p.p.m.): about 3.41 (3H, CH_3O—),

3.93 (3H, CH_3N),

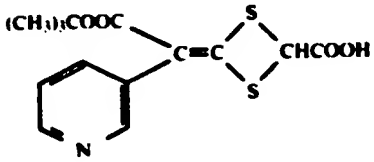
5.15 (1H), 5.24 (1H) } H of C_6 and 

7.39 (1H, 

7.67 (1H, 

8.44 (2H, 

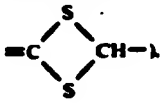
REFERENCE EXAMPLE 20

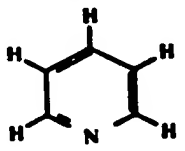


By treating tert-butyl 3-pyridylacetate as in Reference example 15, 4-[(tert-butoxycarbonyl)(3-pyridyl)-methylene]-1,3-dithietane-2-carboxylic acid was obtained.

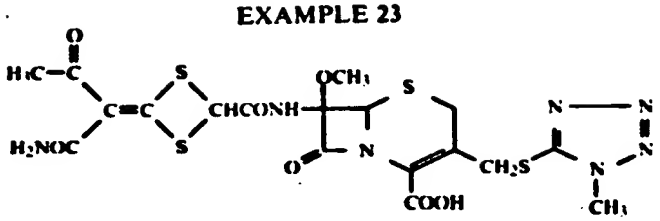
Nuclear magnetic resonance spectra (D_6-DMSO)

δ(p.p.m.): 1.42 (9H, $(CH_3)_3COOC$—),

5.22 (1H, 

7.40 (1H), 7.66 (1H), 8.45 (2H) } 

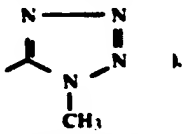
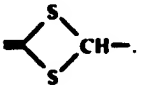
EXAMPLE 23



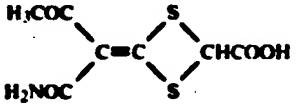
(a) In 55 ml. of anhydrous tetrahydrofuran were dissolved 0.22 g. of 4-[(acetyl)(carbamoyl)methylene]-1,3-dithietane-2-carboxylic acid, 0.496 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester, and 0.194 g. of N,N'-dicyclohexylcarbodiimide. The solution was stirred for 2 hours at room temperature. Insoluble materials were filtered off and the filtrate was concentrated under reduced pressure. The residue formed was subjected to a silica gel column chromatography to provide 0.35 g. of 7β-{4[(acetyl)(carbamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using first a mixture of chloroform and ethyl acetate of 1:1 by volume ratio and then a mixture of chloroform and ethyl acetate of 1:3 by volume ratio as the eluant.

(b). In a mixture of 8 ml. of trifluoroacetic acid and 2 ml. of anisole was dissolved 0.35 g. of the product obtained in the above step (a) at −20° C. followed by stirring for 30 minutes at 0° C. The reaction mixture obtained was concentrated and the residue was mixed with ether followed by stirring for 30 minutes. Then, the precipitates formed were recovered by filtration and washed with ether to provide 0.09 g. of 7β-{[4-(acetyl)(carbamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO)

δ(p.p.m.): 2.32 (3H, ),
3.42 (3H, CH_3O— of C_7),
3.93 (3H, ),
4.30 (2H, —CH_2S— of C_3),
5.17 (1H), 5.26 (1H) } H of C_6 and  .

REFERENCE EXAMPLE 21



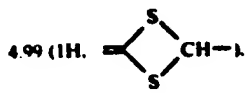
(a). In 50 ml. of tert-butanol was dissolved 5.76 g. of potassium tert-butylate and 50 ml. of anhydrous tetrahydrofuran was added to the solution. Then, after dissolving therein 2.6 g. of acetoacetamide, a solution prepared by dissolving 1.96 g. of carbon disulfide in 5 ml. of anhydrous tetrahydrofuran was added dropwise to the

solution under ice-cooling. To the reaction mixture obtained was added 100 ml. of anhydrous tetrahydrofuran followed by stirring for 1.5 hours at room temperature. Then a suspension prepared by reacting 8 g. of diiodoacetic acid and 1.23 g. of sodium hydride (50% in oil) in 100 ml. of anhydrous tetrahydrofuran under ice-cooling was added to the mixture followed by stirring for 2.5 hours at room temperature.

The reaction mixture obtained was concentrated and the residue was mixed with 50 ml. of 1 normal hydrochloric acid and extracted with 100 ml. of ethyl acetate. The extract was washed with 50 ml. of an aqueous sodium chloride solution and the organic layer formed was extracted with 100 ml. of a saturated aqueous sodium hydrogencarbonate solution. The extract was adjusted to pH 2-3 with concentrated hydrochloric acid and then extracted with 100 ml. of ethyl acetate. The extract was washed with 50 ml. of an aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and concentrated.

The residue formed was dissolved in 30 ml. of methylene chloride and after adding thereto 5 g. of diphenyldiazomethane under ice-cooling, the mixture was stirred for 2 hours at room temperature. The reaction mixture was concentrated and the residue formed was subjected to a silica gel column chromatography to provide 0.6 g. of 4-[(acetyl)(carbamoyl)methylene]-1,3-dithietane-2-carboxylic acid benzhydryl ester using first chloroform and then a mixture of chloroform and methanol of 10:2 by volume ratio as the eluant.

Nuclear magnetic resonance spectra ($CDCL_3$) δ(p.p.m.): 2.32 (3H, H_3COC-),

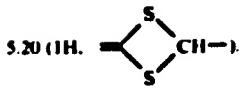


4.99 (1H,

6.97 (1H, —COOC<u>H</u>$(C_6H_5)_2$), 7.2-7.4 (10H, —COOCH(C_6<u>H_5</u>$)_2$).

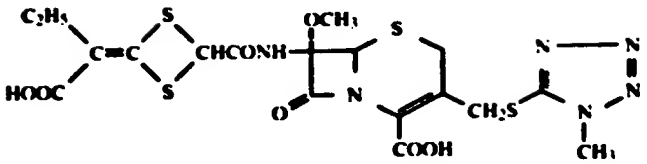
(b). In a mixture of 8 ml. of trifluoroacetic acid and 2 ml. of anisole was dissolved 0.6 g. of the product obtained in the step (a) at −20° C. and the temperature of the reaction mixture was raised to 10° C. over a period of 20 minutes. Then, the reaction mixture was concentrated and 10 ml. of a mixture of ether and petroleum ether of 1:1 by volume ratio was added to the residue to form precipitates, which were recovered by filtration to provide 0.2 g. of 4-[(acetyl)(carbamoyl)methylene]-1,3-diethietane-2-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m): 2.31 (3H, H_3COC—),



5.20 (1H,), 5

EXAMPLE 24



(a) In 15 ml. of tetrahydrofuran was dissolved 610 mg. of 4-(1-tert-butoxycabonylpropylidene)-1,3-diethietane-2-carboxylic acid after adding thereto 1.05 g. of 7β-amino-7α-methoxy-3(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester and 0.5 g. of N,N'-dicyclohexylcarbodiimide, the mixture was stirred for 2 hours at room temperature to cause reaction. After the reaction was over, the N,N'-dicyclohexyl product was filtered off and the solvent was distilled off from the filtrate under reduced pressure to form a caramel-like residue. The residue thus formed was dissolved in 50 ml. of ethyl acetate and the solution was washed with 30 ml. of 1 normal hydrochloric acid and then water and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure to provide 450 mg. of a brown caramel-like residue. The residue was applied to a silica gel column chromatography using 30 g. of silica gel and the product was eluted using first benzene, a mixture of benzene and ethyl acetate of 95:5 by volume ratio, and then a mixture of benzene and ethyl acetate of 90:10 by volume ratio as the eluant, and the fractions containing the product were collected to provide about 10 mg. of 7β-[4-(1-tert-butoxycarboxypropylidene)-1,3-dithietan-2-yl]-caboxamido-7α-methoxy-3(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester.

Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 1.62 (3H, CH_3, t), 2.08 (9H, $(CH_3)_3C-$), 2.66 (2H, $-CH_2-$, q), 4.22 (3H, $-OCH_3$, s), 4.44 (3H, $>N-CH_3$, s),

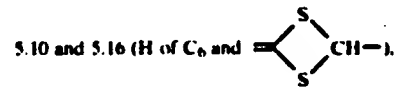
5.40 and 5.47 (H of C_6 and), 7.54 (1H, $-C\underline{H}(C_6H_5)_2$), 7.8-8.2 (10H, $-CH(C_6\underline{H}_5)_2$), 8.30 (1H, —NH—COD—),

(b) In 1.1 ml. of anisole was dissolved 200 mg. of the caramel-like product obtained in the above step and the solution was cooled to about 5° C. in an ice-water bath. To the solution was added dropwise 3.3 ml. of trifluoroacetic acid at a temperature below 10° C. and thereafter the mixture was stirred for one hour at 5°-10° C. to cause reaction. Then, anisole and excessive trifluoroacetic acid were distilled off under reduced pressure at a temperature below room temperature and the residue was mixed with 10 ml. of water and extracted with a mixture of n-butanol and ethyl acetate of 1:1 by volume ratio. The organic layer was collected, washed with a

saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure. The residue was triturated with 10 ml. of ether to provide 54 mg. of 7β-[4-(1-carboxypropylidene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO)

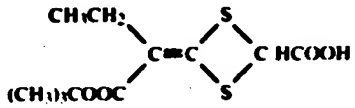
δ(p.p.m.): 0.96 (3H, $-CH_3$, t), 2.00 (2H, $-CH_2-$, q), 3.41 (3H, $-O-CH_3$, s), 3.92 (3H, $>N-CH_3$, s),



5.10 and 5.16 (H of C_6 and =CH—),

9.60 (1H, —NHCO—).

REFERENCE EXAMPLE 22

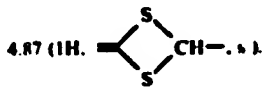


A mixture of 120 ml. of dimethoxyethane and 30 ml. of tetrahydrofuran was cooled to −74° C. in a dry ice-acetone bath and then 4.0 ml. of N-isopropylcyclohexylamine and then 13.72 ml. of a 15% n-butyl lithium n-hexane solution were added to the mixture, whereby the temperature was raised from −73° C. to −62° C. After adding 3.17 g. of tert-butyl butylate and causing reaction for 30 minutes at −74° C. to −75° C., 0.664 ml. of carbon disulfide was added dropwise to the mixture over a period of 10 minutes followed by reaction for 20 minutes at the temperature. Then, 6.86 ml. of a 15% n-butyl lithium n-hexane solution was added dropwise to the reaction mixture at a temperature below −72° C. over a period of 10 minutes and then they were caused to react for 30 minutes. Then, 0.332 ml. of carbon disulfide was added to the reaction mixture over a period of 10 minutes and the reaction was performed for 20 minutes. Furthermore, 3.42 ml. of a 15% n-butyl lithium n-hexane solution was added dropwise to the reaction mixture at a temperature below −72° C. over a period of 13 minutes and then the reaction was further performed for 20 minutes at −74° C. to −73° C. Moreover, 0.166 ml. of carbon disulfide was added to the reaction mixture at about −74° C. over a period of 6 minutes and the reaction was performed for about 25 minutes. Thereafter, sodium diiodoacetate prepared by reacting 0.84 g. of 50% sodium hydride and 5.46 g. of diiodoacetic acid in 25 ml. of dimethoxyethane was added to the reaction mixture followed by reaction for 30 minutes at 0°-5° C. and then the reaction was further continued overnight at room temperature. The solvent was distilled off under reduced pressure from the reaction mixture and the residue was extracted with the addition of 50 ml. of cold ether and 40 ml. of 1 normal hydrochloric acid. The ether layer obtained was

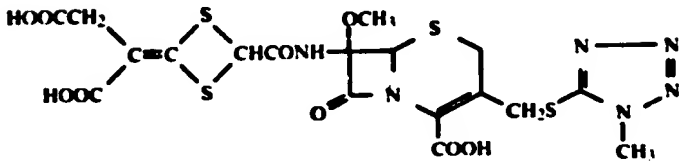
extracted twice each time with 20 ml. of a saturated aqueous sodium hydrogencarbonate solution. The aqueous extracts were combined and adjusted to pH 1 with 1 normal hydrochloric acid, twice with 30 ml. and 20 ml. of ether, successively. The extracts were combined and washed with water, dried over anhydrous magnesium sulfate, and then ether was distilled off to provide 1.08 g. of an oily product. The oily product was applied to a silica gel column chromatography and the fractions containing the product were collected using a mixture of chloroform and methanol of 10:1 by volume ratio to provide 630 mg. of the brown oily 4-(1-carboxypropylidene)-1,3-dithiethane-2-carboxylic acid.

Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 1.24 (3H, $-CH_3$, t), 1.47 (9H, $(CH_3)_3C-$, s), 2.01 (2H, $-CH_2-$, q),



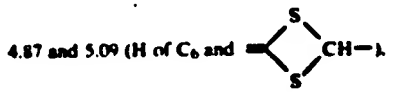
EXAMPLE 25



(a) In 10 ml. of tetrahydrofuran was dissolved 350 mg. of 4-[1,2-bis(tert-butoxycarbonyl)ethylidene]-1,3-dithiethane-2-carboxylic acid. After adding thereto 0.5 g. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester and 219 mg. of N,N'-dicyclohexylcarbodiimide, they were made to react for 2 hours at room temperature. After filtering off the N,N'-dicyclohexylurea thus deposited, the solvent was distilled off from the filtrate, the residue was applied to a silica gel column chromatography and the fractions containing the product were collected using first benzene and then a mixture of benzene and ethyl acetate of 9:1 by volume ratio as the eluant to provide 120 mg. of 7β-}4-[1,2-bis(tert-butoxycarbonyl)ethylidene]-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.42-1.48 (18H, $(CH_3)_3C-$), 2.58 (2H, $-CH_2-$), 3.59 (3H, $-OCH_3$),

3.80 (3H, $>N-CH_3$), 4.36 (2H, $-CH_2-$ of C_2, q),

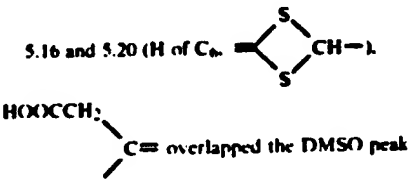


6.92 (1H, $-CH(C_6H_5)_2$), 7.2-7.6 (10H, $-CH(C_6H_5)_2$).

(b) In 1 ml. of anisole was dissolved 115 mg. of the product obtained in the above step (a). After cooling the solution to a temperature below 10° C. in an ice-water bath, 3 ml. of trifluoroacetic acid was added dropwise to the solution at a temperature below 10° C. After stirring the mixture for one hour at 5°-10° C., excess trifluoroacetic acid and anisole were distilled off from the reaction mixture under reduced pressure at room

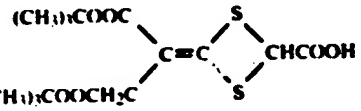
temperature and the residue formed was triturated with 5 ml. of ether to provide 73.7 mg. of 7β-[4-(1,2-dicarboxyethylidene)-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.94 (3H, $>N-CH_3$), 4.30 (2H, $-CH_2-$ of C_2),



$-OCH_3$ overlapped the peak of water.

REFERENCE EXAMPLE 23

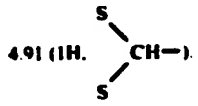


To a mixture of 80 ml. of diethylene glycol dimethyl ether and 20 ml. of tetrahydrofuran was added 1.54 ml. of diisopropylamine. The mixture was cooled to −74° C. with dry ice-acetone bath. Then, 6.86 ml. of a 15% n-butyl lithium n-hexane solution was added to the mixture followed by reaction for 10 minutes at −72° C. to −74° C. Furthermore, 2.3 g. of tert-butyl succinate was added to the reaction mixture and the reaction was further carried out for 30 minutes at −74° C. Then, 0.332 ml. of carbon disulfide was added dropwise to the reaction mixture over a period of about 15 minutes and then the reaction was continued for 15 minutes at −74° C. Also, 3.43 ml. of a 15% n-butyl lithium n-hexane solution was added dropwise to the reaction mixture at a temperture below −71° C. over a period of 20 minutes and the reaction was carried out for 20 minutes at the same temperature. Thereafter, 0.166 ml. of carbon disulfide was added dropwise to the reaction mixture over a period of 13 minutes at a temperature below −72° C. and the reaction was carried out for 17 minutes at the temperature. Moreover, 1.715 ml. of a 15% n-butyl lithium n-hexane solution was added dropwise to the reaction mixture over a period of 10 minutes at a temperature below −71° C. Finally, 0.083 ml. of carbon disulfide was added dropwise to the reaction mixture over a period of 10 minutes and then the reaction was further carried out for 20 minutes at −72° C. to −74° C.

Separately, a suspension of sodium diiodoacetate prepared beforehand from 432 mg. of 50% sodium hydride and 2.8 g. of diiodoacetic acid in 20 ml. of diethylene glycol dimethyl ether dropwise to the reaction mixture obtained in the aforesaid reaction, whereby the

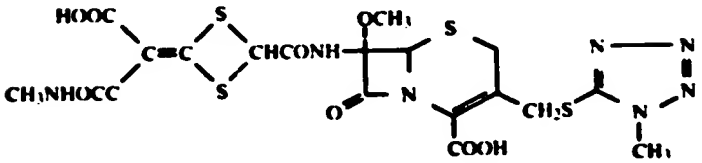
temperature in the system raised from −74° C. to −64° C. Then, the temperature was allowed to raise and after carrying out the reaction for one hour at 0°–5° C., the mixture was stirred overnight at room temperature to cause a further reaction. Thereafter, the solvent was distilled off at room temperature under reduced pressure to provide a brown residue. The residue was mixed with 50 ml. of ether and 20 ml. of a cold 10% sulfuric acid and extracted with ether. The ether layer formed was extracted twice each the time with 50 ml. of a saturated sodium hydrogencarbonate solution. The aqueous layer obtained was mixed with 50 ml. of 10% sulfuric acid and extracted with 50 ml. and 30 ml. of ether, successively. The ether extracts were combined and washed twice each time with 30 ml. of a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and ether was distilled off to provide 1.83 g. of an oily product. The oily product was applied to a silica gel column chromatography using 70 g. of silica gel, eluted using first chloroform and then a mixture of chloroform and methanol of 50:1 by volume ratio, and the fractions containing the product were collected to provide 700 mg. of 4-[1,2-bis(tert-butoxycarbonyl)]-1,3-dithiethane-2-carboxylic acid.

Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.) 1.22 (18H, 2 x $(CH_3)_3C$—), 2.58 (2H, $—CH_2—$),



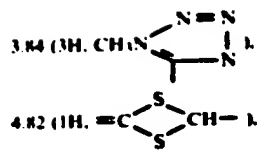
Mass spectra: m/e: 362 M^+

EXAMPLE 26



(a). To a solution prepared by dissolving 500 mg. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester in 35 ml. of tetrahydrofuran was added 500 mg. of 4-[(tert-butoxycarbonyl)-(N-methylcarbamoyl)methylene]-1,3-dithiethane-2-carboxylic acid and about 400 mg. of N,N'-dicyclohexylcarbodiimide, and the mixture was stirred for 3.5 hours at room temperature. The solvent was distilled off from the reaction mixture uner reduced pressure and the residue formed was subjected to a silica gel column chromatography to provide 300 mg. of 7β-{4-[(carboxy)(N-methylcarbamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester using a mixture of chloroform and ethyl acetate of 4:1 by volume ratio as the eluant.

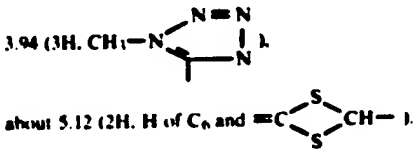
Nuclear magnetic resonance spectra ($CDCl_3$) δ(p.p.m.): 1.52 (9H, t-C_4H_9—), 2.83 (3H, CH_3NHCO-), 3.60 (5H, CH_3O— and $—CH_2—$ of C_2),



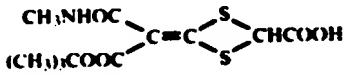
5.08 (1H, H of C_6), 6.93 (1H, $-C\underline{H}(C_6H_5)_2$).

(b). In a mixture of 10 ml. of trifluoroacetic acid and 2 ml. of anisole was dissolved 300 mg. of the product obtained in the above step (a) under ice-cooling. The solution was stirred for about one hour at 15° C. The solvent was distilled off from the reaction mixture under reduced pressure and the residue was mixed with ether followed by stirring. The precipitates thus formed were recovered by filtration and washed with ether to provide 170 mg. of 7β-{4-[(carboxy)(N-methylcarbamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) (p.p.m.): 2.68 (3H, $C\underline{H}_3NHCO$-), 3.42 (3H, CH_3O-),



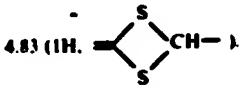
REFERENCE EXAMPLE 24



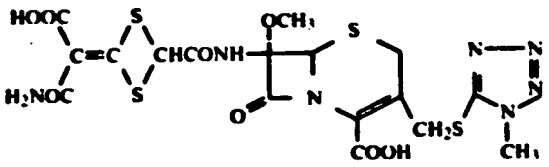
To 3 ml. of 15% potassium tert-butylate tert-butanol solution were added 540 mg. of tert-butyl N-methylmalonamate and 12 ml. of anhydrous tetrahydrofuran with stirring at room temperature. After stirring the mixture for 5 minutes, 0.0935 ml. of carbon disulfide was added dropwise to the mixture followed by stirring for 10 minutes. Then, 1.5 ml. of 15% potassium tert-butylate tert-butanol solution was added to the mixture followed by stirring for 10 minutes, 0.046 ml. of carbon disulfide was added dropwise to the mixture followed by stirring for 10 minutes, and the same procedure was further repeated using 0.8 ml. of 15% potassium tert-butylate tert-butanol solution and 0.023 ml. of carbon disulfide. Then, a suspension of sodium diiodoacetate which was separately prepared by dissolving 0.98 g. of diiodoacetic acid in 7 ml. of anhydrous tetrahydrofuran and adding 115 mg. of 50% sodium hydride to the solution with stirring under cooling, was added to the above reaction mixture followed by stirring for 1 hour at room temperature to complete the reaction.

After the solvent was distilled off under reduced pressure, 150 ml. of ether and 50 ml. of 0.2 normal hydrochloric acid which was cooled at 0° C. were added and the product was extracted with ether, then washed twice each time with 50 ml. of water. Then, ether layer was extracted with 50 ml. of 2% sodium hydrogencarbonate, and the aqueous layer was neutralized to about pH 7.5 with 1 normal hydrochloric acid and extracted with 100 ml. of ether. To the aqueous layer was further added 0.5 ml. of 1 normal hydrochloric acid, and extracted with 100 ml. of ether. This procedure was repeated. Each ether extract was subjected to a silica gel thin layer chromatography using a mixture of acetonitrile, ethylacetate and water of 3:1:1 by volume ratio as the eluant and then fractions containing the product were collected and the solvent was distilled off uner reduced pressure to provide 600 mg. of oily 4-[(tert-butoxycarbonyl)(methylcarbamoyl)-methylene]-1,3-dithietane-2-carboxylic acid.

Nuclear magnetic resonance spectra ($CDCl_3$) (p.p.m.): 1.52 (9H, $(CH_3)_3C$—), 2.84 (3H, CH_3NH-),

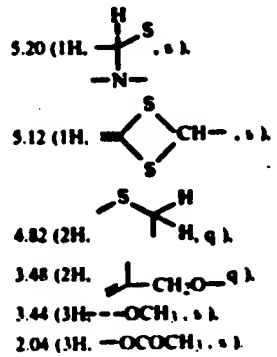


EXAMPLE 27



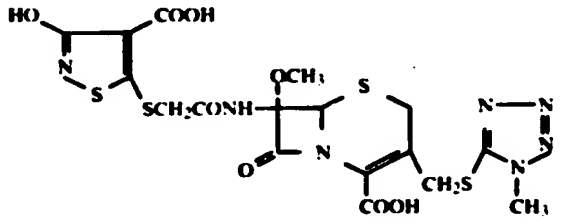
In 8 ml. of an aqueous 5% sodium hydrogencarbonate solution was dissolved 200 mg. of 7β-[4-carboxy-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-cephalosporanic acid. The solution was stirred for 2 hours at room temperature. After the reaction was over, the reaction mixture obtained was adjusted to pH 1 with 2 normal hydrochloric acid and then extracted twice each time with a mixture of n-butanol and ethyl acetate of 1:1 by volume ratio. The organic layer was washed with a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and the solvent was distilled off to provide 180 mg (90% yield) of white-powdery 7β-{4-[(carbamoyl)-(carboxy)methylene]-1,3-dithiethan-2-yl}carboxamido-7α-methoxy-Δ^3-cephalosporanic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) (p.p.m.):



(b) In 6 ml. of water were added 300 mg. of 7β-[4-(carbamoyl)(carboxy)methylene-1,3-dithietan-2-yl]carboxamido-7α-methoxy cephalosporanic acid, 67.2 mg. of 5-mercapto-1-methyltetrazole and 146 mg. of sodium hydrogencarbonate followed by stirring for 16 hours at 60°–62° C. The reaction mixture was adjusted to pH 1 with 2 normal hydrochloric acid under ice-cooling, and the precipitates formed were recovered by filtration, and dried over phosphorus pentoxide under reduced pressure to provide 75 mg. of light yellow powdery 7β-[4-(carbamoyl)(carboxy)methylene-1,3-dithietan-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

EXAMPLE 28

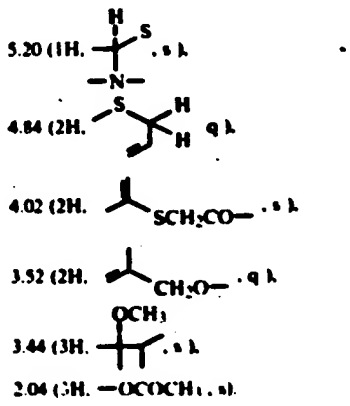


(a) In 60 ml. of methanol was dissolved 6.1 g. of 7β-bromoacetamido-7α-methoxy-cephalosporanic acid.

Then 15 ml. of an ice cooled aqueous solutiion of 4.3 g. of trisodium salt (trihydrate) of 4-carboxy-3-hydroxy-5-mercaptoisothiazole was added dropwise to the solution at 0°–5° C. After stirring the mixture for 30 minutes at the same temperature, methanol was distilled off under reduced pressure. The residue was mixed with 40 ml. of water, adjusted to pH 3 with 2 normal hydrochloric acid, and washed with ethyl acetate. The aqueous layer was further adjusted to pH 1 with 2 normal hydrochloric acid and then extracted twice each time with a mixture of n-butanol and ethyl acetate of 1:1 by volume ratio. The organic layer was washed with a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and the solvent was distilled off to provide a powdery crude product. The product was dissolved in a small amount of methanol and the solution was allowed to cool with ice to form crystals. The crystals were recovered by filtration to provide 4.8 g. (64.2% yield) of the purified white crystals of 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxycephalosporanic acid.

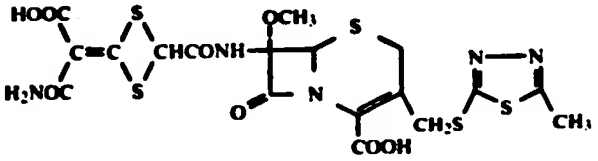
Nuclear magnetic resonance spectra (D_6-DMSO)

δ(p.p.m.):



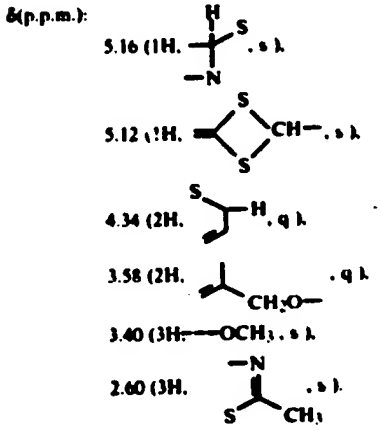
(b) By following the same procedure as in Example 27-b) using 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thioacetoamido-7α-methoxy-cephalosporanic acid and 5-mercapto-1-methyltetrazol, 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thioacetoamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid was obtained.

EXAMPLE 29

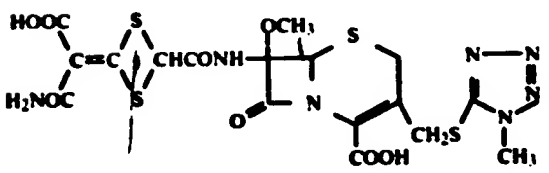


A mixture of 300 mg. of 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxycephalosporanic acid, 76.5 mg. of 2-mercapto-5-methyl-1,3,4-thiadiazole, 146 mg. of sodium hydrogencarbonate, and 6 ml. of water was stirred for 12 hours at 58°–60° C. The reaction mixture was cooled, adjusted to pH 1 with 2 normal hydrochloric acid under ice-cooling, and the precipitates formed were recovered by filtration, and dried over phosphorus pentoxide under reduced pressure to provide 95 mg. (27.8% yield) of light yellow powdery 7β-{4-[(carbamoyl)(carboxy)methylene]-1,3-dithietan-2-yl}-carboxamido-7α-methoxy-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-Δ³-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO)



EXAMPLE 30

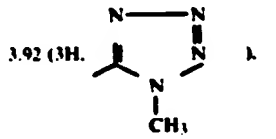


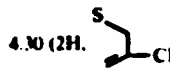
In a mixture of 40 ml. of methanol and 300 ml. of a 5% aqueous sodium hydrogencarbonate solution was dissolved 6.0 g. of 7β-(4-carboxy-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ³-cephem-4-carboxylic acid. The solu-

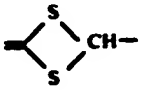
tion was stirred for 5 hours at room temperature. The solution was washed with 300 ml. of ethyl acetate, acidified with diluted hydrochloric acid, and extracted twice each time with 200 ml. of a mixture of n-butanol and ethyl acetate of 1:1 by volume ratio and once with 100 ml. of the same mixture. The organic layers were combined with each other, washed twice each time with 50 ml. of a saturated aqueous sodium chloride solution, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure. To the residue was added 50 ml. of ether and precipitates formed were recovered by filtration, washed with ether and dried to provide crude product. The crude product was purified by a silica gel column chromatography using a mixture of chloroform, methanol, and formic acid of 100:20:1.5 by volume ratio as the eluent.

The fractions containing the product were collected and the solvent was distilled off under reduced pressure to provide 3.5 g. of 7β-{4-[(carbamoyl)(carboxy)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.40 (3H, CH_3O— of C_7), 3.61 (2H, —CH_2— of C_2),

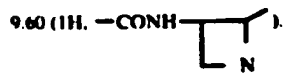




5.11 (1H)
5.14 (1H) } H of C_6 and 

6.00 (1H, —COOH),

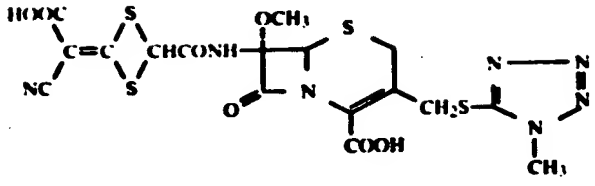
7.50 (2H, —$CONH_2$),



EXAMPLE 31

A mixture of 300 mg. of 7β-(4-carboxy-3-hydroisothiazol-5-yl)thioacetamido-7α-methoxycephalosporanic acid, 67.2 mg. of 5-mercapto-1-methyltetrazole, 146 mg. of sodium hydrogencarbonate, and 6 ml. of water was stirred for 12 hours at 58°–60° C. The reaction mixture was cooled, adjusted to pH 1 with 2 nomral hydrochloric acid in under ice-cooling, and the precipitates formed were recovered by filtration, and dried over phosphorus pentoxide under reduced pressure to provide 7β-{4-[(carbamoyl)(carboxy)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

EXAMPLE 32

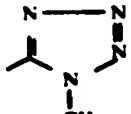


(a). In a mixture of 50 ml. of chloroform and 10 ml. of acetone was suspended 1.0 g. of 7β-{4-[(carbamoyl)-(carboxy)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

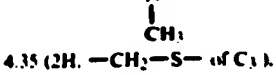
A solution the prepared by dissolving about 700 mg. of diphenyldiazomethane in 5 ml. of chloroform and the solution was added dropwise to the suspension. The mixture was stirred for 30 minutes at room temperature and then the solvent was distilled off from the reaction mixture. The residue was subjected to a silica gel column chromatography using a mixture of chloroform and ethyl acetate of 2:1 by volume ratio as the eluant to isolate and purify the product. Thus, the fractions containing the product were collected and the solvent was distilled off under reduced pressure to provide 0.8 g. of 7β-{4-[(benzhydryloxycarbonyl)(carbamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester.

Nuclear magnetic resonance spectra ($CDCl_3$)

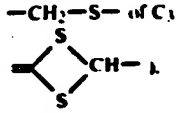
δ(p.p.m.): 3.52 (5H, H of C_2 and CH_3O— of C_7),



3.76 (3H,),



4.35 (2H, $-CH_2-S-$ of C_3),



4.86 (1H,),

5.00 and 5.03 (1H, H of C_6),

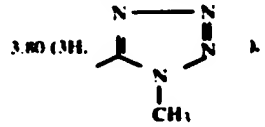
6.90 (1H)
7.00 (1H) } $-CH(C_6H_5)_2$,

7.30 (10H, $-CH(C_6H_5)_2$).

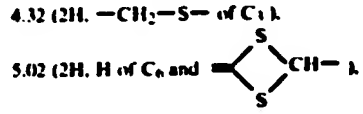
(b). In 10 ml. of chloroform was dissolved 1.0 g. of 7β-{4-[(benzhydryloxycarbonyl)(carbamoyl)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester. While stirring the solution under ice-cooling, 0.3 ml. of pyridine and 0.45 g. of phosphorus pentachloride were added to the solution followed by stirring further for one hour at room temperature. The reaction mixture was then ice-cooled and 3 ml. of water was added to the mixture. The organic layer formed was separated from an aqueous layer, washed with 2 ml. of water, dried over anhydrous magnesium sulfate, and then the solvent was distilled off under reduced pressure to provide 0.5 g. of 7β-{4-[(benzhydryloxycarbonyl)(cyano)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester.

Nuclear magnetic resonance spectra ($CDCl_3$)

δ(p.p.m.): 3.45 (5H, H of C_2 and CH_3O— of C_7),



3.80 (3H,),



4.32 (2H, $-CH_2-S-$ of C_3),

5.02 (2H, H of C_6 and),

6.85 (2H, $-C\underline{H}(C_6H_5)_2$),

9.30 (10H, $-CH(C_6\underline{H}_5)_2$).

(c). In 2 ml. of methylene chloride was dissolved 0.5 g. of 7β-{4-[(benzhydryloxycarbonyl)(cyano)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester. After adding thereto 5 ml. of a mixture of trifluoroacetic acid and anisole in a 4:1 by volume ratio at −10° C., the mixture was stirred for 30 minutes at the same temperature. Then, the solvent was distilled off under reduced pressure at a low temperature from the reaction mixture. The residue was triturated with ether and was recovered by filtration and dried to provide 0.2 g. of powdery 7β-{4-[(carboxy)(cyano)methylene]-1,3-dithietan-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.44 (3H, CH_3O of C_7), 3.62 (2H, H of C_2), 4.30 (2H, $-CH_2-S-$), 5.16 (1H, H of C_6),

5.52 (1H,).

By the same procedure as in Example 1, the following compounds were obtained.

EXAMPLE 33

7β-(4-cyano-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.39 (3H), 3.59 (2H), 3.92 (3H), 4.11 (2H), 4.28 (2H), 5.10 (1H).

EXAMPLE 34

7β-(3-hydroxy-4-phenylisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.40 (3H), 3.56 (2H), 3.87 (2H), 3.92 (3H), 4.27 (2H), 5.05 (1H).

EXAMPLE 35

7β-(3-amino-4-cyanoisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.40 (3H), 3.60 (2H), 3.95 (3H, 4.08 (2H), 4.31 (2H, 5.11 (1H).

EXAMPLE 36

7β-(4-dimethylcarbamoyl-3-hydroxyisothiazol-5-yl)thioacetamido-7β-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 2.88 (6H), 3.38 (3H), 3.56 (2H), 3.90 (5H), 4.26 (2H), 5.04 (1H).

EXAMPLE 37

7β-(3-hydroxyisothiazol-4-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.39 (3H), 3.48 (2H), 3.66 (2H), 3.94 (3H), 4.26 (2H), 5.11 (1H), 7.59 (1H).

EXAMPLE 38

7β-(4-cyano-2-methyl-3-oxo-2,3-dihydroisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.40 (3H), 3.64 (2H), 3.92 (8H), 4.30 (2H), 5.16 (1H).

EXAMPLE 39

7β-[4-cyano-2-(2-hydroxyethyl)-3-oxo-2,3-dihydroisothiazol-5-yl]thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) (p.p.m.): 3.40 (3H), 3.5-3.6 (3H), 3.76 (2H), 3.93 (3H), 4.16 (2H), 4.32 (2H), 5.14 (1H).

EXAMPLE 40

7β-(4-carbamoyl-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) δ(p.p.m.): 3.39 (3H), 3.49 (2H), 3.64 (2H), 3.93 (3H), 4.28 (2H), 5.07 (1H)

EXAMPLE 41

7β-(3-hydroxy-4-hydroxymethylisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6—DMSO) δ(p.p.m.): 3.40 (3H), 3.58 (2H), 3.83 (2H), 3.92 (3H), 4.12 (2H), 4.30 (2H), 5.10 (1H).

EXAMPLE 42

In 7 ml. of methylene chloride was dissolved 300 mg. of 7β-amino-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester, chilled to −30° C. and 460 mg. of pyridine was added. Separately, an acid chloride solution was prepared from the suspension of 240 mg. of potassium (4-cyano-3-methoxyisothiazol-5-yl)thioacetate in 10 ml. of methylene chloride, 170 mg. of oxalyl chloride and a drop of dimethylformamide. The acid chloride solution was added dropwise to the above solution at −30° C. to −20° C. and stirred for one hour at the same temperature. To the reaction mixture was added 30 ml. of chloroform and washed twice each time with 2% hydrochloric acid and twice each time with saturated sodium hydrogencarbonate. The organic layer was then separated and dried over anhydrous magnesium sulfate. The organic layer was condensed under reduced pressure

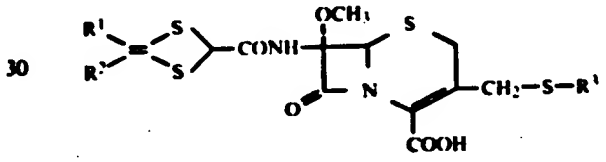
and the residue obtained was subjected to silica gel column chromatography with the eluant of a mixture of chloroform and isopropanol (10:1 by volume ratio). THere was thus obtained 190 mg. of 7β-(4-cyano-3-methoxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid benzhydryl ester.

In 2 ml. of methylene chloride was dissolved the above product and a mixture of 1.6 ml. of trifluoroacetic acid and anisole (3:1 by volume ratio) was added dropwise at −15° C. to −5° C. and stirred for 40 minutes at the same temperature. The solvent was distilled off under reduced pressure, the residue was triturated with 10 ml. of ether, filtered and dried under reduced pressure to provide 120 mg. of a powder of 7β-(4-cyano-3-methoxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid.

Nuclear magnetic resonance spectra (D_6-DMSO) (p.p.m.): 3.40 (3H), 3.58 (2H), 3.92 (3H), 3.99 (3H), 4.15 (2H), 4.18 (2H), 5.12 (1H).

What is claimed is:

1. A 7α-Methoxy-7β-(4-substituted methylene-1,3-dithietane-2-yl) carboxamido-3-heterocyclic thiomethyl-Δ^3-cephem-4-carboxylic acid represented by the formula



wherein R^1 represents a carboxyl group or a derivative thereof selected from the group consisting of lower alkyl, phenyl lower alkyl and naphthyl lower alkyl esters of said carboxyl group and carbamoyl, carbazoyl, and cyano groups; R^2 represents a hydrogen atom, a lower alkyl group, a lower alkoxy group, $R^4S(O)_n$ group wherein R^4 represents a lower alkyl group and n represents 0, 1 or 2, a lower alkanoyl group, a phenyl group, a naphthyl group, a benzoyl group, a naphthoyl group, a R^1 group, a lower alkenyl group, a sulfamoyl group, a pyridyl group or a thiadiazolyl group; and R^3 represents a lower alkyl-substituted tetrazolyl group or a lower alkyl-substituted thiadiazolyl group and the pharmaceutically acceptable salts thereof.

2. 7β-{4-[(Carbamoyl)(carboxy)methylene]-1,3-dithietane-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid as claimed in claim 1.

3. 7β-[4-(1-Carboxyethylidene)-1,3-dithietane-2-yl]carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid as claimed in claim 1.

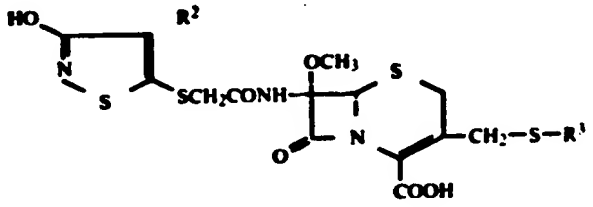
4. 7β-{4-[(Carboxy)(methoxy)methylene]-1,3-dithietane-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid as claimed in claim 1.

5. 7β-{4-[(Carboxy)(methylthio)methylene]-1,3-dithietane-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid as claimed in claim 1.

6. 7β-{4-[(Carboxy)(ethylthio)methylene]-1,3-dithietane-2-yl}carboxamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid as claimed in claim 1.

7. 7β-[4-(Carboxymethylene)-1,3-dithietane-2-yl]carboxamido-7-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid as claimed in claim 1.

8. A 7β-(3-Hydroxy-4-substituted isothiazol-5-yl) thioacetamido-7α-methoxy-3-heterocyclic thiomethyl-Δ^3-cephem-4-carboxylic acid represented by the formula:



wherein R^2 represents a hydrogen atom, a lower alkyl group, a lower alkoxy group, $R^4S(O)_n$ group wherein R^4 represents a lower alkyl group and n represents 0, 1 or 2, a lower alkanoyl group, a phenyl group, a naphthyl group, a benzoyl group, a naphthoyl group, a carboxyl group or a derivative thereof selected from the group consisting of lower alkyl, phenyl lower alkyl and naphthyl lower alkyl esters of said carboxyl group and, carbamoyl, carbazoyl and cyano groups, a lower alkenyl group, a sulfamoyl group, a pyridyl group or a thiadiazolyl group; and R^3 represents a lower alkyl-substituted tetrazolyl group or a lower alkyl-substituted thiadiazolyl group, and the pharmaceutically acceptable non-toxic salts thereof.

9. 7β-(4-Carboxy-3-hydroxyisothiazol-5-yl)thioacetamido-7α-methoxy-3-(1-methyltetrazol-5-yl)thiomethyl-Δ^3-cephem-4-carboxylic acid as claim in claim 8.

* * * * *

ATTACHMENT D

CEFOTAN® (cefotetan disodium)

Chronology of Regulatory Activities

Notice of Claimed Investigational Exemption (IND)

For IND 19,216

09/04/81	Submission to FDA	IND filed
10/08/81	Response from FDA	Acknowledges receipt of IND
10/22/81 11/04/81	Response from FDA	Recommendations and requests following review of IND
12/01/81	Submission to FDA	Response to recommendations and requests
01/21/82 - 09/14/82	Submissions to FDA	Individual protocol submissions
09/20/82	Submission to FDA	Annual Progress Report
09/30/82 - 01/31/83	Submissions to FDA	Individual protocols and amendments
02/07/83	Meeting with FDA	Discussed preclinical issues and clinical plan
02/17/83 - 04/04/83	Submission to FDA	Individual protocols and amendments
04/13/83	Response from FDA	Recommendations and requests following review of IND
04/15/83 - 05/13/83	Submissions to FDA	Individual protocols and amendments
05/11/83	Response from FDA	Additional recommendations and requests following review of IND.
05/18/83 - 08/05/83	Submissions to FDA	Individual protocols and amendments
08/22/83	Submission to FDA	Response to FDA letters of 04/13/83 and 05/11/83. Also, comments stated on preclinical issues raised at 02/07/83 meeting

08/25/83 - 09/16/83	Submissions to FDA	Individual protocols and amendments
09/20/83	Submission to FDA	Annual Progress Report
09/27/83 - 09/10/84	Submissions to FDA	Individual protocols and amendments
10/04/84	Submission to FDA	Annual Progress Report
10/10/84 - 04/11/85	Submissions to FDA	Individual protocols and amendments
04/16/85	Submission to FDA	Clinical trial reports received from ICI UK

New Drug Application (NDA)
For NDA 50-588

01/20/84	Submission to FDA	NDA submitted
01/31/84	Submission to FDA	Revised Table of Contents
04/06/84	Submission to FDA	Amended 01/20/84 submission with HPLC Assay Method
05/29/84	Response from FDA	Recommended changes to package insert
09/21/84	Submission to FDA	Revised package insert
11/19/84	Response from FDA	Revised package insert is accepted
12/20/84	Response from FDA	Cephalosporins - Warnings to be added to labeling
03/21/85	Submission to FDA	Response to 12/20/86 letter
04/03/85	Submission to FDA	Tables to facilitate review of NDA
04/09/85	Submission to FDA	Supplement for surgical prophylaxis
05/21/85	Response from FDA	NDA withdrawn and resubmitted 04/10/85 following 04/09/85 supplement submission

06/05/85	Submission to FDA	Request meeting on 06/14/85 to discuss package insert
06/24/85	Submission to FDA	Revised package insert following 06/14/85 meeting.
07/09/85	Response from FDA	Regarding 01/31/84 and 06/24/85 submissions - 30 days needed to review
08/09/85	Submission to FDA	Final printed labeling
08/26/85	Submission to FDA	Response to telecon - safety update
08/26/85	Submission to FDA	Response to telecon - clinical protocols
10/16/85	Submission to FDA	Response to telecon - technical
10/18/85	Submission to FDA	Response to telecon - preclinical issues
12/27/85	Response from FDA	Approval of NDA

SLR/dmj/6396a